REPORT

of

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., Inc.

1984

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Organization and Policy

The Council for Tobacco Research — U.S.A., Inc. is the sponsoring agency of a program of research into questions of tobacco use and health. It is the outgrowth of an organization formed early in 1954 by tobacco manufacturers, growers and warehousemen. Research support has been mainly through a program of grants-in-aid supplemented by contracts for research with institutions and laboratories. The Council does not operate any research facility.

The Scientific Advisory Board to The Council meets regularly to evaluate applications for research support, judging them solely on the basis of scientific merit and relevance.

The Council awards research grants to independent scientists who are assured complete scientific freedom in conducting their studies. Grantees alone are responsible for reporting or publishing their findings in the accepted scientific manner — through medical and scientific journals and societies.

WILLIAM D. HOBBS
Chairman

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THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., Inc.
900 Third Avenue, New York, N.Y. 10022

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Introduction

In 1984 the Council published the findings of a nine-year study in which mice were exposed to cigarette smoke inhalation.

The results included these: none of the smoke-exposed animals developed pulmonary squamous cell carcinoma; mice pretreated with a pure carcinogen did not have significantly increased lung cancers after prolonged smoke exposure; and the overall numbers of pulmonary neoplasms identified were not statistically significantly different in the smoke-exposed mice, compared to the sham smoke-exposed or shelf control mice.

A summary of the study appears in this report beginning on page 7. It is taken verbatim from the book, "Chronic Exposure of Mice to Cigarette Smoke," which is the final report of scientists at Microbiological Associates, Inc., an independent research company in Bethesda, MD. The work was done there under a contract with the Council, which had the book printed.

In a foreword to the book, Dr. Sheldon C. Sommers, the Council's Scientific Director, wrote: "The experiments represented a determined effort to develop a suitable animal model involving chronic cigarette smoke exposure of inbred mouse strains known to develop various histopathologic types of lung carcinoma after intratracheal administration of pure chemical carcinogens. It is believed the report will be of interest to those engaged in inhalation research and in the field of investigating human smoking and health relationships."

This report contains abstracts of 174 papers that have appeared in the scientific media with acknowledgement of Council support. The figure brings to at least 2,525 the number of scientific papers acknowledging such support published since the Council began supporting research into smoking and health by independent scientists.

As of the end of 1984, the Council's program had provided more than $91,500,000 to 526 investigators for 897 original projects in 285 medical schools, hospitals and research institutions.

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Chronic Exposure of Mice to Cigarette Smoke

Following is the summary section from the book, "Chronic Exposure of Mice to Cigarette Smoke," that comprises the final report of Microbiological Associates, Inc. The section summarizes findings from the 1975-1983 project during which more than 10,000 mice inhaled the smoke from over 800,000 cigarettes.

I. INTRODUCTION

This is the Final Report for Contract 0030 entitled, "Cigarette Smoke Inhalation Studies in Mice." This report describes: 1) the facilities and equipment, 2) the results from three major smoke inhalation studies, and 3) the results from corollary studies that supported the three major programs. The five chapters that make up the Final Report are summarized here.

II. FACILITIES AND EQUIPMENT

The cigarette smoke generation and animal holding equipment were designed to provide high exposure to fresh mainstream cigarette smoke on a daily basis for a major portion of the lifetime of the animals. The facilities provided the following:

- Two Smoke Exposure Machines (SEM II B and C), one sham exposure machine, three animal containment units, twenty-four exposure modules, and 400 animal restraint trays.
- Capacity to expose 480 mice simultaneously, and up to 2000 mice per day, to cigarette smoke under conditions where greater than 90% of the smoke particulates were deposited in the respiratory tract of the mice.
- Documentation of the quantity of smoke presented to the mice during each exposure session.
- Safety systems that assured exposure of mice to smoke only under preset exposure conditions.
- Generation of cigarette smoke under conditions controlled for cigarette type, smoke aerosol concentration and smoke particle size.
- Capacity to house up to 10,000 mice at a time under conditions that insured a clean and controlled environment.

III. CTR-100 (I-100). CO-CARCINOGENESIS AND CHRONIC INHALATION OF 2A1 CIGARETTE SMOKE

The first inhalation study utilized Kentucky Reference 2A1 cigarette smoke (high tar-low nicotine) at a dose of ~1 mg/total particulate matter (TPM) per day per mouse lung. The experiment had four major objectives: 1) to determine the impact of the cigarette smoke generation equipment and delivery system on the test animals (BC3F1/Cum female mice), 2) to determine the toxicological effects of daily exposure to 2A1 whole cigarette smoke in BC3F1/Cum mice over a 12–15 month period, 3) to characterize the susceptibility of BC3F1/Cum and C3H/Anf/Cum mice to lung cancer after treatment with known chemical carcinogens, and 4) to determine the effects of daily exposure to whole cigarette smoke on 3-methylcholanthrene (MCA)-induced lung tumors. A total of 3476 mice were put on test for this study.

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Mice were observed during and immediately after daily smoke and sham treatment. For the smoke exposed animals, while a certain amount of adaptation occurred, the animals never accepted the smoke exposure without some agitation or struggling in the holder. They also occasionally demonstrated some shallow breathing or gasping during exposure. Immediately after exposure, mice in the smoke exposed groups were lethargic, ataxic, and hypothermic. The sham exposed animals also demonstrated agitation and struggling while restrained in the holders, but generally appeared normal after exposure. Carboxyhemoglobin (COHb) levels in the smoke exposed mice averaged between 40 and 50% after each exposure session. The results from the 2A1 smoke, sham, and shelf control groups will be presented first, followed by the results from the MCA-treated group.

The impact of the exposures and related manipulations resulted in ~50% of the animals dying in the smoke or sham exposed groups by 36 weeks on test. In addition, neck abrasions were observed in both smoke and sham exposed mice. Two equipment modifications were recommended: temperature compensated flow thermistors to monitor smoke/air flow through the modules and polycarbonate inserts to cushion the neck edge of the restraint. The thermistors were designed, developed, and utilized. Polycarbonate neck inserts were designed but their utilization was not approved.

Data from all sham exposed controls showed that the spontaneous incidence of malignant lung tumors in CTR-100 (all alveolar adenocarcinomas) was 0.5%. Smoke exposed animals were observed to have no malignant lung changed during the study. The most frequent lesion observed in the smoke exposed groups was pigmented alveolar macrophage accumulation (PAMA). After 20 weeks of exposure, the incidence of PAMA was 100%. Two other lesions were associated with smoke exposure, squamous metaplasia of the trachea and rhinitis of the nose.

Treatment of BC3F1/Cum mice with MCA resulted in ~62 different combinations of histopathologic diagnoses in the lung. Alveologenic lesions were classified into 4 categories: alveolar hyperplasia (AH), alveolar noncompressing nodules (ANCN), alveolar compressing nodules (ACN), and alveolar adenocarcinoma (AAC). Squamous lesions were classified into 3 categories: squamous metaplasia (SM), squamous neoplasm (SN), and squamous cell carcinoma (SCC). The other carcinomas found included poorly differentiated carcinomas (PDC) and a variety of mixed carcinomas. The carcinomas metastasized and eventually caused the death of the animal. The progressive nature of these lesions was documented and a scheme depicting the evolution of these malignancies was postulated (see Figure 29 and Section III.D). Ancillary studies with other known carcinogens and other strains of mice suggested: 1) C3H/Anf Cum and BC3F1/Cum mice were identical in their susceptibility to MCA-induced lung cancers; 2) a dose response to MCA induced lung tumors was observed; and 3) the carcinogenic activity for lung tissue was determined to be greatest for MCA, followed by 7,8-dihydro-7,8-dihydroxy benzo(a)pyrene (BaP-7,8-diol), both of which were much more active than benzo(a)pyrene (BaP).

Daily exposure to 2A1 cigarette smoke did not alter the types of MCA-induced pulmonary lesions observed compared to sham exposed mice. There were slight variations in the incidence and distribution of the lesions as a function of duration of smoke exposure. Analysis of individual lung lesions showed that the MCA-sham exposed group had a higher incidence and/or shorter latency for all malignant lung tumors than the MCA-smoke exposed group. Smoke exposed mice developed a slightly higher incidence of ANCN, ACN, and/or SN than the sham exposed mice. Analysis of the development of either these latter lesions *or* malignant lesions was also performed. Daily exposure to 2A1 cigarette smoke did not alter the incidence and/or distribution of these MCA-induced lesions when compared to the sham exposed controls.

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IV. CTR-101A. CHRONIC EXPOSURE OF BC3F1/CUM MICE TO 2R1 CIGARETTE SMOKE

This second inhalation study was the first designed to use long-term or "lifetime" chronic smoke exposure. Future studies were to address such variables as specie sensitivity, smoke exposure regimen, smoke dose, cigarette type, and duration of exposure. However, after initiation of this second study, it was decided not to perform any other long-term smoke inhalation studies. Thus, the main objective of this second inhalation study was to determine the potential biological activity of daily exposure to Kentucky Reference 2R1 cigarette smoke (high tar-high nicotine) under one specific set of experimental conditions. A secondary objective was the determination of the possible effect of daily exposure to 2R1 cigarette smoke on benzo(a)pyrene-induced lung tumors.

The number of mice originally put on test for the smoke, sham and shelf groups was 2053, 1014 and 449, respectively. BaP was given intratracheally to another group of mice. The number of mice put on test for the BaP + smoke, BaP + sham, and BaP + shelf groups was 320, 260, and 130, respectively. BaP was given three times at biweekly intervals and smoke/sham exposure was initiated one week after the last BaP treatment. Smoke exposure continued on a daily basis for 110 weeks. The dose of 2R1 cigarette smoke was determined to be 0.2 mg TPM per day per mouse lung. The results from the smoke, sham and shelf control groups will be repesented first, followed by the results from the BaP-treated groups.

The major clinical signs in the smoke exposed, sham exposed, and shelf control mice were as follows. 1) The major reasons for death of the mice were either conditions that randomly removed animals from the study (i.e. smoke or sham exposure-related, holder-related, or documented air or smoke flow problems), or developing diseases that likely led to the death of the animal. 2) Rate of weight gain was slower in the sham and smoke groups compared to the shelf controls. 3) Daily COHb levels averaged 17.2%, 1.4% and 1.8% for the smoke, sham and shelf groups, respectively.

Among the shelf controls, 60% of the mice died of such neoplastic diseases as hematopoietic cancers (~33%) and fibrosarcomas (~13%), while 40% died of non-neoplastic lesions such as congestion/pneumonia (~8%), nephritis (~4%), and a variety of incidental lesions (~23%). These same types of lesions were observed in the smoke and sham exposed mice. Detailed analyses of the following lesions were made in smoke and sham exposed animals: 1) lung cancers, 2) non-neoplastic respiratory tract lesions, 3) other non-neoplastic lesions, 4) all malignancies, 5) head and neck fibrosarcomas, and 6) hematopoietic tumors.

All lung cancers observed in the BC3F1/Cum mice were alveolar adenocarcinomas (AAC). No squamous cell carcinomas or other pulmonary carcinomas were observed in the smoke, sham, or shelf control groups. A total of 19 AAC were observed out of a total of 978 animals at risk in the smoke exposed group, while 7 AAC were observed out of 651 sham exposed animals at risk. Data analysis (see Section IV. H.) in four different ways indicated that no differences were observed between the smoke or sham exposed groups at $p \leq 0.05$. The incidence of AAC was greater in the smoke exposed groups compared to the sham exposed groups ($p = 0.17$) and the latency of expression was decreased in the smoke exposed groups compared to the sham exposed groups ($p = 0.10$). Occurrence of ANCN or ACN was 38/978 for smoke exposed groups and 21/651 for the sham exposed groups [$p = 0.51$ (incidence); $p = 0.38$ (latency)]. The occurrence of either AAC or ACN yielded the largest difference between the smoke and sham exposed groups. The final incidence of AAC or ACN was 49/978 in the smoke exposed group compared to 28/651 in the sham exposed group.

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These incidences were not different ($p = 0.59$), but the tumors appeared more quickly in the smoke exposed group ($p = 0.09$).

A total of five non-neoplastic respiratory tract lesions were analyzed: PAMA, congestion, rhinitis, otitis media, and otitis externa. The overall incidence of PAMA was 16% which is considerably lower than that observed in the previous cigarette smoke study. The number of PAMAs increased with exposure, but only reached 35% during the last 4 months of exposure. Congestion was a rather common lesion in both the smoke and sham exposed animals. The occurrence of congestion was greater in the sham exposed animals compared to the smoke exposed mice ($p = 0.02$). The final incidence of rhinitis was not different between the smoke and sham exposed groups ($p = 0.97$), but the lesion occurred slightly more rapidly in the smoke exposed mice ($p = .26$). The latency period for otitis media was shorter in smoke exposed mice $p \leq 0.01$) and according to one analysis, the final incidence of this lesion was also higher in smoke exposed animals ($p = 0.05$). The final incidence of otitis externa was not different between the smoke and sham exposed groups ($p = 0.45$). However, there were indications that this lesion occurred with a shorter latency in the smoke exposed group compared to the sham exposed group ($p = 0.10$).

The other non-neoplastic lesion observed was nephritis, an inflammatory lesion of the kidneys. There were no differences between the smoke exposed and sham exposed groups.

A comparison of the occurrence of all malignancies demonstrated a 27% incidence for the smoke exposed and a 29% incidence for the sham exposed animals. The incidence and/or latency was not different between the two groups.

One particular type of tumor, head and neck fibrosarcoma, was observed with a higher frequency in the smoke exposed mice compared to the sham exposed mice. These tumors were highly aggressive and metastasized to tissues far removed from the primary site. A total of 29 head and neck fibrosarcomas were observed in the smoke exposed mice, compared with 8 in the sham exposed groups. This difference is highly significant ($p \leq 0.05$). Exposure to smoke was associated with both a higher incidence and shorter latency of head and neck fibrosarcomas. The mechanism by which these tumors developed is unclear.

Hematopoietic tumors included lymphosarcomas, reticulum cell sarcomas, and lymphocytic or lymphoid leukemias. The total incidence of these tumors was 13% in the smoke exposed and 19% in the sham exposed animals. Using four different methods of analysis, the incidence of these tumors was greater, and the latency period was shorter in the sham exposed compared to the smoke exposed animals ($p \leq 0.05$).

Analysis of the BaP-treated groups was performed as described above. The incidence of PAMA in the BaP + smoke exposed group was 51%. For the animals exposed to 2R1 smoke without BaP treatment, the incidence of PAMA was less than 20%. It would seem that treatment with BaP modified the macrophage response resulting in higher incidence of these pigmented cells in the lung. Daily exposure to 2R1 cigarette smoke during the time BaP-induced lung tumors developed did not alter the incidence or latency in the BaP + smoke groups compared to the BaP + sham exposed groups.

V. CTR-101B. EXPOSURE OF BC3F1/CUM MICE TO 2R1 CIGARETTE SMOKE

During 1979-1980, an exposure regimen was developed that resulted in low toxicity for both 2R1 and 3A1 cigarette smoke. These exposure conditions allowed the scheduling of a long term study whereby the biological effects of smoke from both

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cigarette types could be simultaneously evaluated. Such a long term study employing this smoke exposure regimen was proposed, approved, and initiated during the 1980 contract year. In July 1980, however, it was decided that this study should not be completed and that smoke exposure under these newly defined conditions would be limited to the length of time during which animals in CTR-101A were to be exposed to smoke. The experiments conducted in the CTR-101B were designed to evaluate certain short-term toxicological endpoints that may be relevant to determining the potential biological activity of whole cigarette smoke.

The smoke exposure regimen for in this study did not include rest periods between successive cigarettes, used an exposure period of 15 seconds per minute, and continuous exposure for 17 "runs" in 140 minutes. This regimen resulted in ~80 ug TPM deposited per day per mouse lung, an average COHb of 35.9%, and greater than 95% incidence of PAMA by 16 weeks of daily smoke exposure.

The following assays were selected for their potential to predict or monitor long-term effects of cigarette smoke: 1) inhibition of pulmonary DNA repair in vitro; 2) stimulation of pulmonary DNA synthesis in vitro; 3) induction of pulmonary aryl hydrocarbon hydroxylase (AHH) activity; 4) induction of ornithine decarboxylase (ODC) activity; 5) augmentation of the DNA damaging effects of particular lung carcinogens; 6) induction of sister chromatid exchange (SCE) in bone marrow; 7) alteration in certain physical characteristics such as lung weight (wet and dry), body weight, and biochemical characteristics such as DNA, protein and hydroxyproline levels in lung, 8) alteration of immune competence, and 9) alteration in rate of atresia (i.e. oocyte toxicity).

Using the discontinuous exposure regimen as defined in CTR-100, inhibition of DNA repair capacity [e.g. unscheduled DNA synthesis (UDS)] occurred within 13 weeks of daily exposure to 2A1 or 3A1 cigarette smoke, but was unaffected by exposure to 2R1 cigarette smoke for even up to 52 weeks. Using the continuous regimen as defined for CTR-101B, no DNA repair inhibition was observed for either 3A1 or 2R1 cigarette smoke even after 52 weeks of daily exposure. Thus, the inhibition of DNA repair (UDS) by cigarette smoke would seem to be dependent upon cigarette type (i.e. 2A1, 3A1 $\gg$ 2R1) and/or the exposure regimen (i.e. 30 seconds smoke per minute $\gg$ 15–20 seconds smoke per minute).

Replicative DNA synthesis (RDS) in vitro was stimulated 2- to 4-fold after 9 weeks of exposure to 2A1 or 3A1 cigarette smoke, but not after exposure to 2R1 cigarette smoke using the high dose, conventional exposure regimen. RDS was stimulated ~2-fold in mice exposed to either 2R1 or 3A1 cigarette smoke using the continuous exposure regimen.

DNA replication as measured by tritiated thymidine incorporation into lung cells in vivo [Labelling Index (LI)], was increased 3- to 6-fold in lung and tracheal tissues from 2R1 and 3A1 cigarette smoke exposed mice. No increase in LI was observed in bladder, colon, liver, kidney, or spleen. Evidence was presented to suggest that daily exposure to whole smoke (either 2R1 or 3A1), while stimulating normal DNA replication, may induce unscheduled DNA synthesis or repair synthesis as well. Daily exposure to 2R1 or 3A1 cigarette smoke failed to augment the LI induced by the potent lung carcinogen, BaP-7,8-diol. Thus, both cigarette smoke and certain chemical carcinogens (e.g. BaP-7,8-diol) were capable of stimulating DNA synthesis in pulmonary tissue, but these effects did not seem to be additive, synergistic, or antagonistic.

AHH was induced in the lung after exposure to 2R1 or 3A1 cigarette smoke using any exposure regimen investigated. Pulmonary AHH activity was stimulated 4- to 5-fold after 3, 6, or 9 months exposure. Renal AHH was induced after daily exposure for 9 months. Hepatic AHH was uneffected at all time intervals.

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Pulmonary ODC activity, a potential marker for promotion, was induced ~2-fold within 3 hours after daily exposure to 2R1 or 3A1 smoke for 3 or 6 months.

Exposure to 2R1 or 3A1 cigarette smoke failed to cause single strand breaks in the DNA of pulmonary cells. Smoke exposure also failed to influence the level of DNA damage caused by BaP or BaP-7,8-diol.

Sister chromatid exchange (SCE) was induced in bone marrow cells of mice after exposure to either 2R1 or 3A1 smoke. The induction was ~2-fold and was first observed after 1 week smoke exposure. Continued exposure for 4, 12 or 46 weeks showed no further increase in the number of SCEs. Increases in SCEs persisted after cessation of smoke exposure for at least 1 week, whether the mice were exposed initially for 1 week or 46 weeks ($p \leq 0.05$).

Exposure to either 2R1 or 3A1 cigarette smoke using the continuous regimen resulted in an increase in the cellularity of pulmonary tissue. Daily exposure from 1 week to 52 weeks caused a time-dependent increase in lung wet weight, lung dry weight, lung DNA and protein, and lung hydroxyproline. This increase in tissue mass was likely the result of increased cellular proliferation. This proliferation was probably a final manifestation of the increased DNA synthesis rates observed in these animals.

Daily exposure to 2R1 and 3A1 cigarette smoke using different exposure regimens failed to alter the immune status of BC3F1/Cum mice. Immune competence was determined by Jerne plaque assay. Neither acute nor chronic exposure caused immunosuppression in either BC3F1/Cum or BALB/c Cum mice.

Rates of atresia (i.e. natural loss of oocytes from the ovary) were also uneffected by chronic exposure to 2R1 or 3A1 cigarette smoke. While it has been suggested in the literature that there is a relationship between cigarette smoking and early onset of menopause (i.e. when the ovaries are depleted of oocytes), oocyte toxicity was not observed in BC3F1/Cum mice after 52 weeks of daily smoke exposure.

VI. COROLLARY STUDIES: PHARMACOKINETICS OF INHALED MATERIALS

The deposition, distribution, and clearance were determined for nicotine, BaP, dotriacontane (DTC) and catechol in cigarette smoke. Data showed considerable differences among these four classes of chemicals. Nicotine, BaP and catechol showed rapid redistribution from the lung to other tissues and clearance from the body. The half-life for internal retention (t1/2) was less than 15 minutes for nicotine, less than 1 hour for BaP, and less than 20 minutes for catechol. DTC showed little redistribution from the lung, with a t1/2 of greater that 24 hours. The deposition, distribution and clearance of catechol as single chemical aerosol was shown to be identical to catechol in cigarette smoke—i.e. the pharmacokinetics of catechol are not altered by the presence of the other chemicals in cigarette smoke. Greater than 90% of the inhaled catechol, either in cigarette smoke or as an aerosol, was found as a beta-glucuronidase- or aryl sulfatase-sensitive conjugate in the urine within two hours of exposure.

The deposition, distribution, and clearance was also determined for 12-0-tetradecanylphorbol-13-acetate (TPA) as a single chemical aerosol. The pharmacokinetics of aerosolized TPA was investigated as a potential promoter in a two-stage (initiation and promotion) carcinogenesis model system specific for lung tissue. Results from two experiments showed that over 65% of the initial TPA deposition was found in the respiratory tract. TPA was rapidly cleared from the lung via the blood, with increasing

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accumulation in the liver, stomach, and intestines up to 8 hours after exposure. By 48 hours, over 70% of the inhaled TPA was found in the feces and 20% in the urine. Aerosolized TPA induced a specific biochemical event in the lungs, that is, induction of ODC activity, within 3 hours after inhalation. TPA thus exerted its effect immediately and was then removed from the lung. The pharmacokinetics data suggested that aerosolized TPA may have effects in the gastrointestinal tract, in addition to the respiratory tract. These data supported the use of TPA in a two-stage model for lung carcinogenesis.

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Abstracts of Reports

Following are abstracts, approved by the authors, of reports on new research acknowledging support from The Council that have appeared in scientific journals since publication of the 1983 Report. The name of the grant recipient is in italics.

The abstracts are grouped under these headings: I. Cancer-Related Studies, II. The Respiratory System, III. Heart and Circulation, IV. Neuropharmacology and Physiology, V. Pharmacology and Biochemistry, VI. Immunology and Adaptive Mechanisms, VII. Metabolic Studies, VIII. Epidemiology.

I. *Cancer-Related Studies*

HIGH-DENSITY LIPOPROTEINS DECREASE BOTH BINDING OF A POLYNUCLEAR AROMATIC HYDROCARBON CARCINOGEN TO DNA AND CARCINOGEN-INITIATED CELL TRANSFORMATION

Adsorptive endocytosis of lipoproteins (LP), specified by LP apoprotein binding to receptors on the cell membrane coated-pit regions, is proposed as a mechanism for internalization of LP-bound lipophilic compounds into cells. Transport of lipophilic compounds, such as benzo (α) pyrene (BaP), is reported to be a function of serum LP *in vivo*, and to represent a potential mechanism for movement of BaP from the portal of entry site to other tissues. In the presence of high-density lipoproteins, a mutagenic BaP metabolite was bound to cellular DNA at decreased levels when compared to cells exposed to the mutagen in the absence of high-density lipoproteins. Fetal calf serum with low levels of lipoproteins, specifically high-density lipoproteins, is associated with efficient methylcholanthrene-initiated transformation of C3H/10T½ cells, while calf serum with a significant concentration of high-density lipoproteins requires up to a 500% increase in methylcholanthrene concentration to achieve similar levels of transformation in this mouse embryo cell line. When concentrated serum lipoproteins or purified HDL were added to fetal calf serum containing MCA at 1μg/ml, the C3H/10T½ transformation frequency was decreased compared to the transformation frequency achieved in the presence of 1μg/ml of MCA in fetal calf serum without supplementation. These results suggest that high-density lipoprotein partitioning of lipophilic polynuclear aromatic hydrocarbon mutagens from the cell culture medium may effectively reduce the concentration of carcinogen available for interaction with cellular DNA *in vitro*, which, in turn, may be associated with decreased carcinogen-induced transformation of cells.

Busbee, D. L. and Benedict, W. F.

Mutation Research III: 429-439, 1983.

Other support: American Cancer Society and National Institutes of Health.

From the Genetics Center, Department of Biological Sciences, North Texas State University Denton; and the Division of Hematology-Oncology, Los Angeles Children's Hospital, and Department of Pediatrics, University of Southern California School of Medicine, Los Angeles.

HUMAN LYMPHOCYTES TREATED WITH *r*-7, *t*-8-DIHYDROXY-*t*-9,10-EPOXY-7,8,9,10-EPOXY-7,8,9,10-TETRAHYDROBENZO[α]PYRENE REQUIRE LOW-DENSITY LIPOPROTEINS FOR DNA EXCISION REPAIR

In the present *in vitro* studies, human lymphocytes which were nonmitogen-

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stimulated and depleted of lipoproteins, were found to be deficient in DNA excision repair typically initiated in these cells in response to treatment with a direct-acting polynuclear aromatic hydrocarbon carcinogen. Lymphocytes either depleted of lipoproteins or supplemented with human low-density lipoproteins formed DNA-carcinogen adducts which were not chromatographically distinguishable. The state of lipoprotein depletion did not alter lymphocyte uptake of thymidine from the medium. Lymphocytes which were depleted of lipoproteins, treated with carcinogen, and subsequently supplemented with low-density lipoproteins, regained the ability to engage in DNA excision repair. The data suggest that either low-density lipoprotein(s), or a component(s) of low-density lipoprotein(s), is required by human lymphocytes in order to initiate excision repair of carcinogen-damaged DNA.

Joe, C. O., Rankin, P. W., and *Busbee, D. L.*

Mutation Research 131:37-43, 1984.

Other support: American Cancer Society and a North Texas State University Faculty Research Grant.

From the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station; U.S. Department of Agriculture, Agricultural Research Service, Veterinary Toxicology and Entomology Research Laboratory, College Station; Genetics Center, Department of Biological Sciences, North Texas State University, Denton.

BENZO[α]PYRENE UPTAKE BY LYMPH: A POSSIBLE TRANSPORT MODE FOR IMMUNOSUPPRESSIVE CHEMICALS

A study of benzo[α]pyrene (BaP) uptake into sheep lymph lipoproteins is presented in this paper as a potential model for explaining the exposure of immune system cells to high concentrations of xenobiotic compounds. Both absorption of BaP into the lymphatic circulation from the gastrointestinal system, and BaP partitioning into lymph lipoproteins *in vitro* have been examined. Results showed that BaP, a lipophilic promutagen, reached maximal concentrations in the thoracic duct lymphatic circulation within two hours after gastric instillation. BaP in lymph obtained by thoracic duct canulation decreased to approximately control levels within four hours after treatment. When lymph was not allowed to enter the blood vascular circulation, serum levels of BaP increased very slowly, suggesting minimal mesenteric blood vascular absorption of the lipophilic hydrocarbon. BaP partitions into lymph lipoproteins as a function of the lipoprotein concentration. The data suggest that low-density lipoproteins may take up BaP more efficiently than do very low-density or high-density lipoproteins, and that lymph components other than lipoproteins do not take up and transport BaP. The authors propose that lipophilic xenobiotic compounds interact with cells of the immune system via lymphatic lipoprotein transport of potentially mutagenic, carcinogenic, or immunosuppressive agents.

Busbee, D. L. et al.

Journal of Toxicology and Environmental Health 13:43-51, 1984.

Other support: National Heart, Lung and Blood Institute, American Cancer Society, and the U. S. Department of Agriculture.

From the Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station.

INHIBITION OF DNA S
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Busbee, D.L. et al.

Proceedings of the Nati
81:5300-5304, 1984.

Other support: National
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INHIBITION OF DNA SYNTHESIS BY AN ELECTROPHILIC METABOLITE OF BENZO[α]PYRENE

The results of a study of DNA synthesis inhibition by (±)-*anti*-(7*r*,8*t*)-dihydroxy-(9,10*t*)-epoxy-7,8,9,10-tetrahydrobenzo[α]pyrene (BPDE) are presented in this paper. It can be seen that mitogen-stimulated scheduled DNA synthesis and DNA excision repair in human lymphocytes, as well as DNA polymerase α activity in a cell-free system, were inhibited by this electrophilic metabolite of benzo[α]pyrene, which covalently binds to cellular macromolecules and is mutagenic, carcinogenic, and cytotoxic. Specifically, human lymphocytes treated with BPDE at concentrations greater than 500-800 ng/ml showed decreases in both mitogen-stimulated DNA synthesis and excision repair of damaged DNA but did not exhibit overt cytotoxicity. Formation and total concentration of BPDE-DNA adducts were not correlated with inhibition of DNA synthesis. DNA polymerase α studies using a cell-free system showed that enzymatic activity was not diminished when purified polymerase was treated with BPDE prior to the addition of template DNA. When the template DNA concentration was varied, BPDE inhibition of enzyme activity was uncompetitive. BPDE inhibition of enzyme activity was found to be noncompetitive when concentrations of dATP, dCTP, or dTTP were varied, but competitive when the concentration of dGTP was varied. The date indicate that BPDE competitively inhibits interaction of dGTP with the template-DNA polymerase α complex.

Busbee, D.L. et al.

Proceedings of the National Academy of Sciences of the United States of America 81:5300-5304, 1984.

Other support: National Institutes of Health, U.S. Department of Agriculture and the Texas Agriculture Experiment Station.

From the Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station; U.S. Department of Agriculture, Agriculture Research Service, Veterinary Toxicology and Entomology Research Laboratory, College Station, TX; and the Department of Biological Science, North Texas State University, Denton.

BENZO(α)PYRENE UPTAKE BY SERUM LIPIDS: CORRELATION WITH TRIGLYCERIDE CONCENTRATION

An *in vitro* study of the relationship between benzo(α)pyrene (BaP) association with serum lipoproteins (LP) and LP composition was conducted using human subjects. BaP partitioning into different serum LP ranged from 53 to 71% of available BaP. Efficiency of BaP partitioning into serum LP was examined to determine if there was a relationship between partitioning into LP and the lipid components of LP. The data indicate that triglyceride (TG) concentrations were more directly correlated with LP partitioning of BaP than were concentrates of other LP components. Adjusting sera to a uniform TG concentration (96.5 mg/dl) resulted in the same BaP uptake for each serum type, while adjusting sera to contain a uniform cholesterol concentration (104.6 mg/dl) did not result in similar BaP uptake among serum types. Analysis of serum LP composition suggested that marked differences in both BaP uptake and serum TG concentrations among the subjects were due mainly to differences in serum very low

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

density lipoprotein (VLDL) concentrations. A correlation study using 14 human subjects showed that serum TG concentration was the best predictor ($r = 0.973$, $P < 0.001$) for BaP uptake by serum, followed by phospholipid ($r = 0.685$, $P < 0.01$) and total cholesterol ($r = 0.514$, $P < 0.05$) concentrations. The results indicate that serum TG concentration (typically VLDL-TG) may be the primary factor affecting BaP uptake by serum LP, and suggest that a small change in serum TG concentration could cause a significant increase in BaP uptake by serum LP, contributing to an increased level of circulating carcinogen.

Yoo, J.S.H., Norman, J.O., and *Busbee, D.L.*

Proceedings of The Society for Experimental Biology and Medicine 177:434-440, 1984.

Other support: National Institutes of Health, U.S. Department of Agriculture and the Texas Agricultural Experiment Station.

From the Departments of Anatomy and of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station; Veterinary Toxicology and Entomology Research Laboratory, United States Department of Agriculture, College Station, TX.

A RELATIONSHIP BETWEEN THE YEAST CELL CYCLE GENES CDC4 AND CDC36 AND THE *ets* SEQUENCE OF ONCOGENIC VIRUS E26

Reported here is significant primary sequence homology among the predicted translational products of three genes: CDC4, CDC36, and *ets*. CDC4 and CDC36 are *Saccharomyces cerevisiae* cell division cycle genes, while *ets* is a transformation-specific sequence of avian erythroblastosis virus E26. The deduced primary structures of the three gene products were compared by computer to a large data base of known and predicted protein sequences. The search revealed 22.0-22.5% identity over regions of 140-206 codons, respectively, between the different pairwise combinations. For these particular sequences, these identity scores fall 3.4-4.0 standard deviations above the empirically-determined mean values of fortuitous similarity. *S. cerevisiae* cells require CDC36 and CDC4 in order to complete two early events in the cell cycle: execution of start (CDC36) and spindle pole body separation (CDC4). In virus E26, the *ets* sequence is linked in frame with Δgag and myb^E in the tripartite structure 5′-Δgag-myb^E-*ets*-3′, comprising the E26 transforming oncogene. The homologies described here suggest that the biochemical functions or regulation of the CDC4, CDC36 and *ets* products may be related.

Peterson, T.A., Yochem, J., Byers, B., Nunn, M.F., *Duesberg, P.H.*, Doolittle, R.F., and Reed, S.L.

Nature 309(5968):556-558, 1984.

Other support: National Institutes of Health, National Science Foundation and the American Cancer Society.

From the Department of Biological Sciences, University of California, Santa Barbara; Department of Genetics, University of Washington, Seattle; Department of Molecular Biology and Virus Laboratory, University of California, Berkeley; and the Department of Chemistry, University of California, San Diego, La Jolla.

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Nunn, M. F., Seeburg,

Nature (London) 306:3

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TRIPARTITE STRUCTURE OF THE AVIAN ERYTHROBLASTOSIS VIRUS E26 TRANSFORMING GENE

There are only two avian oncogenic viruses that specifically cause acute leukemias yet do not transform chicken fibroblasts in culture: (1) E26, which causes erythroblastosis and a low level of concomitant myeloblastosis in chickens, and (2) avian myeloblastosis virus (AMV), which causes myeloblastosis exclusively. Both viruses are replication-defective and share a sequence termed *myb* (also known as *amv*) which is unrelated to essential virion genes and is therefore thought to be part of the transforming *onc* genes of these viruses. However, the genetic structure of the two viruses differ. E26 has a genomic RNA of 5.7 kilobases (kb) and encodes a 135,000 molecular weight *gag*-related protein (p135) with probable transforming function. The investigators show here by *in vitro* translation that the 5.7-kb E26 RNA directs the synthesis of p135. Oligonucleotide analysis indicates that E26 RNA contains an internal 0.8-kb subset of the 1.2-kb AMV-related sequence (myb^A), termed myb^E. A 2.46-kb molecular clone prepared from cDNA transcribed *in vitro* from E26 RNA contained an E26 transformation-specific (*ets*) sequence flanked by myb^E and an *env*-related sequence. A complete DNA sequence of this clone indicates that the 1.5-kb *ets* sequence extends the open reading frame of myb^E for 491 amino acids. Thus, the p135 gene of E26 is a genetic hybrid of three distinct elements, ~1.2 kb derived from the 5′ region of the retrovirus *gag* gene, myb^E and the *ets* sequence, linked in the order 5′-Δ*gag*-myb^E-*ets*-3′. The myeloid leukemogenicity shared by E26 and AMV correlates with the common *myb* sequence, while the distinct erythroid leukemogenicity of E26 correlates with *ets* and the E26-specific linkage of *myb* to Δgag.

Nunn, M. F., Seeburg, P. H., Moscovici, C., and *Duesberg, P. H.*

Nature (London) 306:391-395, 1983.

Other support: National Institutes of Health and the Medical Research Service of the Veterans Administration.

From the Department of Molecular Biology and Virus Laboratory, University of California, Berkeley; Genentech, Inc., South San Francisco; and the Virus Research Laboratory, Veterans Administration Hospital, Gainesville, FL.

QUANTITATION OF FIBRONOGEN INFLUX AND FIBRIN DEPOSITION AND TURNOVER IN LINE 1 AND LINE 10 GUINEA PIG CARCINOMAS

The data presented here provide a quantitative analysis of fibrinogen influx and fibrin accumulation and turnover in line 1 and line 10 carcinomas. Specifically, radiolabeled guinea pig fibrinogen (GPF) was used to measure fibrinogen influx and fibrin accumulation in line 1 and line 10 hepato- (bile duct) carcinomas growing in the s.c. space of syngeneic strain 2 guinea pigs over the course of 7 days following transplant. This time course represents an interval of growth uncomplicated by immunological tumor rejection or by significant tumor necrosis. Earlier immunofluorescence studies revealed fibrin deposits in both tumors with line 1 ≫ line 10. In accord with these data, GPF accumulated in both tumors in amounts that matched or exceeded plasma fibrinogen levels. Line 1 tumor GPF content was 4-fold greater than that of line 10 tumors and 11-33-fold that of normal s.c. tissue. The composition of tumor fibrinogen-fibrin was investigated by aqueous and urea extraction. The fraction of total accumulated GPF that was urea insoluble, and therefore presumably cross-linked fibrin, was constant over time but strikingly different for line 1 (65%) and line 10 (48%) tumors, as com-

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

pared with control s.c. tissue (18%). By 7 days, line 1 tumors (mean weight, 0.77 g) contained nearly 2 mg of fibrinogen-fibrin, and line 10 tumors (mean weight, 0.62 g) contained nearly 0.5 mg. Influx of GPF and initial clotting were constant over time and equivalent for the two tumors. Hence, the large differences in GPF accumulation observed between these tumors apparently reflect differences in fibrinolysis, not in fibrinogen influx or coagulation. The data presented indicate substantial traffic of plasma fibrinogen into and out of both tumors, as compared with control tissues, equivalent to nearly 10 and 7 ml of plasma over 7 days of growth for line 1 and line 10 tumors, respectively; comparable values for normal s.c. tissues were 1.0 to 1.4 ml plasma fibrinogen.

Dvorak, H. F., Harvey, V. S. and McDonagh, J.

Cancer Research 44:3348-3354, 1984.

Other support: U. S. Public Health Service and the National Foundation for Cancer Research.

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston.

CHEMICAL-VIRAL INTERACTIONS IN CELL TRANSFORMATION

At the beginning of this basic science review, it is noted that the carcinogenic process is the consequence of a complex interaction between diverse environmental and host factors. Although the underlying mechanisms have not been delineated, early animal studies have demonstrated that chemical carcinogens could enhance neoplasia resulting from exposure to known oncogenic viruses. A significant technical advance which permits a more critical analysis of the molecular basis of carcinogen-viral synergy has been the development of well-defined cell culture systems responsive to multiple factors in transformation. In the case of DNA viruses such as SV40 and adenovirus, preexposure of the target cells to the carcinogen has usually been found to be a prerequisite for enhancement of viral transformation. This temporal relationship has been interpreted as indicating that DNA damage and repair may be important mediators of this enhancement. However, the ability of carcinogens to induce transformation in RNA tumor virus infected cells has been found to require a different temporal application of agents than in the DNA virus systems. In addition to being multifactor, there is now evidence from several *in vivo* systems that the carcinogenic process also involves several stages in its development. In the two-stage mouse skin assay system, two qualitatively different stages have been identified, one termed "initiation" and the other "promotion." Cell culture systems may represent valuable model systems for analyzing both the multifactor and multistep nature of carcinogenesis. Sections of this paper are devoted to Enhancement of Viral Transformation by Initiating Chemical Carcinogens; Enhancement of Viral Transformation by Tumor Promoting Agents and Related Compounds; Progression of the Transformed Phenotype; and Multistep Model of Adenovirus Transformation.

Fisher, P. B.

Cancer Investigation 1(6):495-509, 1983.

Other support: National Cancer Institute and the American Cancer Society.

From the Department of Microbiology, Columbia University College of Physicians & Surgeons, New York.

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ACCELERATED ONSET OF VIRAL TRANSCRIPTION IN ADENOVIRUS-INFECTED HeLa CELLS TREATED WITH THE TUMOR PROMOTER 12-*O*-TETRADECANOYL-PHORBOL-13-ACETATE

Diterpene phorbol ester tumor promoters, such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), increase the transformation frequency of cells infected with type 5 adenovirus (Ad5), simuan virus 40, polyoma, Epstein-Barr virus, and bovine papilloma virus. In a number of virus-transformed cells, exposure to TPA has been shown to increase the amount of viral RNA, and in uninfected cells, TPA rapidly induces the synthesis of specific RNAs and polypeptides. To test the hypothesis that accelerated production of Ad5 mRNA resulted from early onset of transcription, the investigators measured incorporation of [^{3}H]uridine into viral RNA during a 5-min pulse at various times after infection at 33°C. Early viral RNA was detected in TPA-treated cells 2 to 3 h before it was detected in samples from control cultures. Specific radioactivities of RNA from treated and control cells were similar at each time point, suggesting that increased labeling of viral RNA did not result from changes in transport of [^{3}H]uridine or from alterations in the UTP pool size or availability that favored mRNA synthesis nonspecifically. It was also noted that when Ad5-infected HeLa cells were exposed to the tumor promoter TPA, acceleration was not dependent upon concurrent protein synthesis and could not be attributed to rapid entry of virus into the cell nucleus. TPA suppressed the transcription-delay phenotype of the E1a mutant, hrl, without restoring its ability to replicate.

Carter, T. H., Milovanovic, Z. Z., Babiss, L. E., and *Fisher, P. B.*

Molecular and Cellular Biology 4(3):563-566, 1984.

Other support: National Cancer Institute and the National Institute for Allergy and Infectious Diseases.

From the Department of Biological Sciences, St. John's University, Jamaica, NY, and the Department of Microbiology, Cancer Center, College of Physicians & Surgeons, Columbia University, New York.

DELETION AND INSERTION MUTATIONS IN EARLY REGION 1a OF TYPE 5 ADENOVIRUS THAT PRODUCE COLD-SENSITIVE OR DEFECTIVE PHENOTYPES FOR TRANSFORMATION

The early region 1a (E1a) gene of adenovirus 5 has been shown to serve an essential role in initiating both the viral productive cycle in permissive cells and the transformation of rodent cells. On the basis of earlier findings showing that H5hrl (hrl) is cold sensitive for transformation, a series of mutants were constructed so that they contained deletions or insertions in different sites of E1a to ascertain: (i) whether the cold-sensitive phenotype of hrl was the result of the identified single-base pair deletion of nucleotide 1,055 or due to a missense mutation at another site and (ii) what region and how much of the E1a 51-kilodalton protein are actually required to produce cell transformation. A mutant, H5d1101 (d1101), was constructed to contain a 5-base pair deletion of nucleotides 1,008 to 1,012, which produced a frameshift and a subsequent stop codon at nucleotide 1,241. This mutant, which should encode a truncated 33-kilodalton protein in place of the wild-type 51-kilodalton protein, had a cold-sensitive phenotype for transformation essentially identical to hrl. Consonant with this finding, a mutant (H5in106) engineered to contain a 16-base pair insertion initiated after nucleotide 1,009

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

with a stop codon beginning at the newly inserted nucleotide 1,013, also had a cold-sensitive phenotype like hrl and d1101. It is striking, however, that a mutant (H5d1105) with a 69-base pair deletion beginning at nucleotide 1,003 and having a stop codon at nucleotide 1,544, was totally defective for transformation at any temperature. Transfection studies with plasmids containing the Ela or Ela and Elb genes of sub309, hrl1, and d1101 further revealed that the cold-sensitive transformation phenotype observed could be exhibited in the absence of viral Elb gene expression.

Babiss, L. E., *Fisher, P. B.*, and Ginsberg, H. S.

Journal of Virology 49(3):731-740; 1984.

Other support: National Institute of Allergy and Infectious Diseases.

From the Department of Microbiology, Cancer Center/Institute of Cancer Research, College of Physicians & Surgeons, Columbia University, New York.

MUTATIONAL ANALYSIS OF THE FUNCTIONAL DOMAINS OF THE Ad5-Ela GENE PRODUCTS AFFECTING CELL TRANSFORMATION

The studies described in this paper deal with identifying and understanding the type 5 adenovirus Ela and Elb gene products that are necessary to initiate and maintain the transformed cell phenotype. Using both Ad5hrl and additional viral mutants containing insertion or deletion mutations affecting the coding capacity of the Ela 13 S mRNA, the importance of the viral 51 kd polypeptide is demonstrated for maintaining the transformed cell phenotype and its possible interactions with the Ela 12 S gene product. A cloned rat embryo fibroblast cell line (CREF) was used for all the studies described here. The heart of this paper is divided into four major sections: (1) the CREF Cell-Adenovirus Transformation Assay; (2) Transformation Studies Using Ad5hrl and CREF Cells; (3) Construction of Ela Deletion and Insertion Mutants, and (4) Transformation Characteristics of sub309 and Ela Deletion and Insertion Mutants. In summary, the data described using viruses with specific mutations in the portion of the genome encoding the Ela 51 kd protein imply that it is the viral gene product that is necessary to maintain the recognized properties of transformed cells. The conditionally lethal, cold-sensitive phenotype expressed by the mutated genomes, whether introduced into CREF cells by infection with virions or by transfection with intact genomes or plasmids, implies that the Ela 51 kd protein interacts with a host macromolecule on the Ela 48 kd polypeptide. The cold-sensitive phenotype may reflect a decreased binding constant at 32°C between the mutants' truncated proteins and a still unidentified host and/or viral macromolecule. Identification of the putative host component involved and the characteristics of the viral protein-host macromolecule interaction should reveal critical clues to understanding the mechanisms of viral transformation.

Babiss, L. E., *Fisher, P. B.* and Ginsberg, H. S.

In: Ginsberg, H. S. and Vogel, H. J. (eds.): *Transfer and Expression of Eukaryotic Genes*, New York: Academic Press, 1984, pp. 247-262.

Other support: National Institute of Allergy and Infectious Diseases.

From the Department of Microbiology and Comprehensive Cancer Center, College of Physicians & Surgeons, Columbia University, New York.

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ENHANCEMENT OF VIRAL TRANSFORMATION AND EXPRESSION OF THE TRANSFORMED PHENOTYPE BY TUMOR PROMOTERS

In the summary of this wide-ranging review of carcinogenesis, it is noted that any comprehensive model attempting to describe the molecular events involved in carcinogenesis must incorporate the presently available information on the various phases of the carcinogenic process, i.e., initiation, promotion and progression. The first event in chemical carcinogenesis, termed "initiation," is believed to involve the covalent binding of a metabolically activated or direct acting carcinogen to cellular DNA. This interaction with target DNA can result in a permanent change in DNA sequences as a result of point mutations, or it could result in a genetically altered cell by inducing changes in the expression of defined DNA sequences without altering the structure of DNA. In the special case of individuals exhibiting cancer-prone syndromes, a large population of initiated cells may be present at birth and a further increase in genetically altered cells may develop as a result of subsequent exposure to initiating carcinogens in the environment. Although not clearly defined on a biochemical or molecular level, the subsequent events in carcinogenesis appear to involve clonal outgrowth of an initiated cell population. This process, termed "promotion," has been studied in numerous *in vivo* and *in vitro* systems. The complexity of the promotion phenomenon has recently been emphasized by the observation that there may be several stages in promotion. The final stage in the neoplastic process, termed "progression," involves the development of clonal variants that have acquired qualitatively different phenotypes from the original initiated cell population or display enhanced expression of the transformed state. With the development of both *in vivo* and *in vitro* model systems that exhibit the "progression phenomenon," future studies may provide a more precise molecular understanding of the process by which a tumor cell evolves into a metastatic neoplasm.

Fisher, P. B.

In: Slaga, T. J. (ed.): *Mechanisms of Tumor Promotion: volume III, Tumor Promotion and Carcinogenesis In Vitro*, Boca Raton, FL, CRC Press, 1984, pp. 57-123.

Other support: National Cancer Institute and the American Cancer Society.

From the Department of Microbiology, Columbia University College of Physicians & Surgeons, New York.

EXPRESSION CLONING OF HUMAN EGF RECEPTOR COMPLEMENTARY DNA: GENE AMPLIFICATION AND THREE RELATED MESSENGER RNA PRODUCTS IN A431 CELLS

Epidermal growth factor (EGF), a peptide of 53 amino acids derived from a 128-kD precursor predicted by recombinant DNA analysis, effects both growth and induction of specific differentiated functions in target tissues. In order to define more fully the mechanisms by which polypeptide growth factors regulate gene transcription and cellular growth, expression cloning techniques were used in this study to select human EGF receptor complementary DNA clones. The EGF 3′ coding domain shows striking homology to the transforming gene products of avian erthyroblastosis virus. Overexpression of EGF receptors in A431 cell lines correlates with increased EGF receptor mRNA levels and amplification (up to 110 times) of the apparently singular EGF receptor gene. There are three cytoplasmic polyadenylated RNA products of EGF

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

receptor gene expression in A431 cells, one of which contains only 5′ (EGF binding domain) sequences and is postulated to encode the secreted EGF receptor-related protein. According to these authors, the use of expression cloning and an RNA sandwich screening approach has made available EGF receptor cDNA which permits experiments designed to evaluate structure-function relations between receptor-ligand interaction, specific gene transcription, and growth regulation. These studies also demonstrate that amplification of a picto-oncogene determines production of a growth factor receptor and cellular responses to this growth factor.

Lin, C. R. Chen, W. S., Kruiger, W., Stolarsky, L. S., Weber, W., Evans, R. M., Verma, I. M., *Gill, G. N.*, and Rosenfeld, M. G.

Science 224:843-848, 1984.

Other support: National Institutes of Health, American Cancer Society, Deutsche Forschungsgemeinschaft, and the Dutch Queen Wilhemina Fund.

From the Eukaryotic Regulatory Biology Program, University of California, San Diego, School of Medicine, La Jolla; Molecular Biology and Virology Laboratory, The Salk Institute, La Jolla, CA, and the Division of Endocrinology, University of California, San Diego, School of Medicine, La Jolla.

HUMAN ADENOVIRUS 2 E1B-19K and E1B-53K TUMOR ANTIGENS: ANTIPEPTIDE ANTIBODIES TARGETED TO THE NH_2 AND COOH TERMINI

In this study, antibodies that were prepared against synthetic peptides 8 to 16 amino acids in length, encoded at the HN_2 and COOH termini of the major E1B-19K and E1B-53K tumor antigens. The antipeptide antibodies immunoprecipitated the targeted E1b-19K or E1B-53K tumor antigens from extracts of Ad2-infected cells. The specificity of the antipeptide antibody recognition of the E1B tumor antigens was confirmed by peptide competition studies. Antipeptide antibodies directed to the NH_2 and COOH termini immunoprecipitated the E1b-53K tumor antigens from two Ad2-transformed rat cell lines, F17 and F4, providing evidence that identical tumor antigens are synthesized in Ad2-infected and Ad2-transformed cells. These results show that the E1b-19K and E1b-53K T antigens are not processed proteolytically at either the Nh_2 or COOH terminus. The data provide strong evidence at the protein level that the E1B-19K and E1B-53K tumor antigens partially overlap in DNA sequence, with the E1B-19K initiating translation at the first ATG at nucleotide 1711 in translation reading frame 1 and the E1b-53K tumor antigen initiating translation at the second ATG at nucleotide 2016 in reading frame 3. Overall, these data establish at the protein level the minimum gene boundaries, translation-initiation sites, and reading frames of the Ad2 E1B-19K and E1B-53K genes, thus confirming theoretical deductions based on DNA sequence and peptide map analyses. The antipeptide antibodies should prove useful for studies on the purification and functions of the Ad2 E1B-19K and E1B-53K T antigens.

Green, M. et al.

Journal of Virology 48(3):604-615, 1983.

Other support: U. S. Public Health Service.

From the Institute for Molecular Virology, St. Louis University Medical Center, St. Louis.

ANTIBODIES TO SYNT
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Green, M. et al.

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Lucher, L. A., Brackm

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

ANTIBODIES TO SYNTHETIC PEPTIDES TARGETED TO THE TRANSFORMING GENES OF HUMAN ADENOVIRUSES: AN APPROACH TO UNDERSTANDING EARLY VIRAL GENE FUNCTION

This extensive paper starts with a brief review of recent studies using antipeptide antibodies to investigate problems in virology. A general strategy is then outlined which uses antipeptide antibodies to investigate the known Ad early proteins and to detect putative, as yet unidentified, proteins whose existence is suggested by the presence of open translation reading frames as predicted from the Ad DNA sequence. Many particular studies are then described which can be identified by the following section titles: Genetic Organization of the Ad2 Genome: Potential Sites for Targeting Antipeptide Antibodies; Early Viral Gene Expression; Late Viral Gene Expression; Adenovirus-Transforming Genes: Organization, Gene Products, and Functional Studies; Molecular Characterization of Proteins Encoded by Adenovirus-Transforming Genes Using Antipeptide Antibodies; Organization of Ad2 E1 Region; Studies Using Antipeptide Antibodies Targeted to the Ad2 E1B 53K, 19K, and 20K Proteins; Use of Antipeptide Antibodies to Analyze the Ad2 Large Plaque Mutants that Are Defective in Cell Transformation; Use of Antipeptide Antibodies to Identify Ad2 and Ad12 E1A-Encoded Proteins in Infected and Transformed Mammalian Cells and in *E. coli* Cells Transformed by E1A cDNA-Containing Peptides, and Projections on Adenovirus-Transforming Proteins. In this last section, establishing the functions of the adenovirus early proteins, and in particular the early region E1-encoded transforming proteins, is presented as a challenging and exciting task, since investigators can optimistically look forward to the availability of microgram quantities of purified E1 proteins for further studies.

Green, M. et al.

Current Topics in Microbiology and Immunology 109:167-191, 1983.

Other support: National Cancer Institute.

From the Institute for Molecular Virology, St. Louis University Medical Center, St. Louis.

ANTIBODY DIRECTED TO A SYNTHETIC PEPTIDE ENCODING THE NH_2-TERMINAL 16 AMINO ACIDS OF THE ADENOVIRUS TYPE 2 E1B-53K TUMOR ANTIGEN RECOGNIZES THE E1B-20K TUMOR ANTIGEN

As reported here, a peptide, H_2N-Glu-Arg-Arg-Asn-Pro-Ser-Glu-Arg-Gly-Val-Pro-Ala-Gly-Phe-Ser-Gly-(Cys)COOH, containing the amino acid sequence at the NH_2 terminus of the adenovirus type 2 (Ad2) E1B-coded large T antigen (E1B-53K) has been synthesized. Antipeptide antibody was generated in rabbits and used to immunoprecipitate Ad T antigens from Ad2 early infected cell extracts. In addition to the expected E1B-53K T antigen, antipeptide antibody precipitated the Ad2 E1B-20K T antigen that was previously shown to be related to E1B-53K (*Green, M. et al., J. Virol.* 42, 30-41, 1982). Antipeptide antibody prepared against the COOH terminus of the E1B-53K T antigen or against the NH_2 terminus of the E1B-19K T antigen did not precipitate the E1B-20K T antigen. These data suggest that the Ad2 E1B-20K T antigen initiates translation at nucleotide 2016 in reading frame 3, as does E1B-53K. The viral mRNA that encodes the E1B-20K T antigen has not been identified.

Lucher, L. A., Brackmann, K. H., Symington, J. S., and *Green, M.*

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Virology 132:217-221, 1984.

Other support: National Institutes of Health.

From the Institute for Molecular Virology, St. Louis Medical Center, St. Louis.

EPSTEIN-BARR HR-1 VIRION DNA IS VERY HIGHLY METHYLATED

It is well established that DNA methylation is centrally involved in gene expression. A very important question now is how tumor viruses regulate their genes in the lytic as well as in the integrated state. In the study presented here, EBV DNA from the HR-1 strain, a nontransforming variant, was produced and purified three times. The final purified virus preparation was lysed with protease K (100 μg) and sodium dodecyl sulfate (1%). After several other preparatory steps, the 5-methylcytosine content of virion DNA of EBV and its host HR-1 cells was analyzed. 5-methylcytosine is the only methylated base in eukaryotic DNA. The bases were measured after formic acid hydrolysis by high-performance liquid chromatography. The percentage of cytosines methylated in the EBV HR-1 virion DNA was extremely high, averaging close to 15% for three determinations. DNA methylation of the host Burkitt's lymphoma cell line HR-1 was much lower, averaging 2.48% for 12 determinations. This extent of methylation is in great contrast to other classes of tumor viruses and is the highest that is known in any mammalian system. The striking new observation that EBV DNA is methylated to such a large extent raises the question of how methylation may regulate gene expression in this virus. According to these authors, it is essential that future experiments determine which genes are methylated and to what extent methylation occurs in the virion and intracellularly.

Diala, E. S. and *Hoffman, R. M.*

Journal of Virology 45(1):482-483, 1983.

Other support: National Cancer Institute.

From the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla.

THE EXTENT OF DNA METHYLATION IN HUMAN TUMOR CELLS

In this study, the total genomic DNA methylation, *i.e.*, the percentage of methylated cytosines, was measured in 20 cell lines derived from different types of human tumors. The measurements were obtained by cation-exchange liquid chromatography of bases released by formic acid hydrolysis. These experiments were done to determine if altered methylation is a prevalent and large defect in oncogenic transformation. A majority of the tumor cells measured had decreased levels of methylated DNA in comparison to this and other laboratories' published measurements of normal cells and tissues. In fact, tumor cell DNA ranged as low as 1.2% of cytosines methylated compared to a value of 3% or more for normal cells and tissues. HpaII and MspI DNA restriction enzyme analysis confirmed for all tumor cell lines tested that their DNA was hypomethylated in comparison to the DNA from normal diploid fibroblasts tested. The results obtained by liquid chromatography and restriction enzyme analysis were strikingly similar. The reduced methylation of the tumor cell DNA correlated with the

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Diala, E. S., Cheah

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Other support: Nati
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recent observation of other laboratories that individual genes are undermethylated in human cancer cells and that a number of different carcinogens can lower DNA methylation directly.

Diala, E. S., Cheah, M. S. C., Rowitch, D., and *Hoffman, R. M.*

Journal of the National Cancer Institute 71(4):755-764, 1983.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla.

S-ADENOSYLMETHIONINE SYNTHETASE IN CULTURED NORMAL AND ONCOGENICALLY-TRANSFORMED HUMAN AND RAT CELLS

In this attempt to recognize differences in the metabolism of methionine and homocysteine in normal and transformed cells, the enzymatic formation of S-adenosylmethionine was examined in extracts of a variety of normal and oncogenically-transformed human and rat cell lines which differ in their ability to grow in medium in which methionine is replaced by its immediate precursor homocysteine. These investigators have localized the bulk of the S-adenosylmethionine synthetase activity to the post-mitochondrial supernatant. They show that in all cell lines a single kinetic species exists in a dialyzed extract with a K_m for methionine of about 3-12μM. In selected lines they have demonstrated a requirement for Mg^{2+} in addition to that needed to form the Mg·ATP complex for enzyme activity and have shown that the enzyme can be regulated by product feedback inhibition. Because the investigators detect no differences in the enzymatic ability of these cell extracts to utilize methionine for S-adenosylmethionine formation *in vitro*, they suggest that the failure of oncogenically-transformed cell lines to grow in methionine-free, homocysteine-containing medium may result from the decreased methionine pools in these cells or from the loss of ability of these cells to properly metabolize homocysteine, adenosine or their cellular product, S-adenosylhomocysteine.

Oden, K. L., Carson, K., Mecham, J. O., *Hoffman, R. M.*, and Clarke, S.

Biochimica et Biophysica Acta 760:270-277, 1983.

Other support: National Institutes of Health, the American Heart Association and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, and the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla.

REDUCED FREE-METHIONINE IN METHIONINE-DEPENDENT SV40-TRANSFORMED HUMAN FIBOBLASTS SYNTHESIZING APPARENTLY NORMAL AMOUNTS OF METHIONINE

In the long-running search for distinct metabolic differences between normal and cancer cells, many different types of cancer cells have been shown to be methionine-dependent. These cells, unlike normal cells, grow poorly or not at all when methionine

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

is replaced by its immediate precursor homocysteine in the growth medium (Met Hcy⁺ medium). These investigators have previously shown that apparently normal total amounts of methionine are synthesized by methionine-dependent SV40-transformed human fibroblasts. However, methionine-dependent cells in Met Hcy⁺ medium accumulate reduced amounts of S-adenosylmethionine (AdoMet) and elevated amounts of S-adenosylhomocysteine (AdoHcy) that together probably limit growth. In this report, the investigators demonstrate that the amount of free methionine is low in methionine-dependent SV40-transformed human fibroblasts in Met Hcy⁺ medium compared to normal human diploid fibroblasts. In contrast, in Met⁻ Hcy⁺ medium, the amount of free methionine is comparable in both cell types. The deficient pool of free methionine in methionine-dependent cells in Met⁻ Hcy⁺ medium allows only low amounts of AdoMet to be formed. However, large amounts of biosynthesized methionine are channeled into protein synthesis. Possible mechanisms are discussed to explain this cancer-associated metabolic defect.

Stern, P. H., Mechan, J. O., Wallace, C. D., and *Hoffman, R. M.*

Journal of Cellular Physiology 117:9-14, 1983.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla.

THE METABOLIC DEFECT OF METHIONINE DEPENDENCE OCCURS FREQUENTLY IN HUMAN TUMOR CELL LINES.

A metabolic characteristic or defect present in cancer cells but not in normals may shed light on the molecular basis of oncogenic transformation. This paper deals with such a characteristic. Methionine dependence is the inability of cells to grow when methionine (Met) is replaced by its immediate precursor homocysteine (Hcy) in the culture medium Met⁻ Hcy⁺ medium). All normal unestablished cell strains tested to date have been shown to be methionine-independent and thus grow almost as well in Met⁻ Hcy⁺ medium as they do in Met⁺ Hcy⁺ medium. Results presented here indicate that out of 23 cell lines derived from diverse types of human tumors, 11 do not grow at all in Met⁻ Hcy⁺ medium and are absolutely methionine-dependent and three grow only slightly in this medium. Many of the tumor cell lines tested have little else in common other than the fact that they are methionine-dependent. The high frequency of occurrence of methionine dependence in diverse types of human tumor cells indicates that methionine dependence may be an important aspect of oncogenic transformation and therapeutically exploitable.

Mecham, J. O., Rowitch, D., Wallace, C. D., Stern, P. H., and *Hoffman, R. M.*

Biochemical and Biophysical Research Communications 117(2):429-434, 1983.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla.

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Other support: National C
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ALTERED METHIONINE METABOLISM OCCURS IN ALL MEMBERS OF A SET OF DIVERSE HUMAN TUMOR CELL LINES

This biochemical study examines the biochemical basis of methionine dependence, a metabolic defect found thus far only in transformed and malignant cells. The defect is manifested as the inability of cells to grow in media in which methionine (Met) is replaced by its immediate precursor homocysteine (Hcy) (Met Hcy⁺ media). It is demonstrated here that methionine-dependent cells derived from human tumors, compared to normal methionine-independent cells, have low levels of free Met, low levels of S-adenosylmethionine (AdoMet) and elevated levels of S-adenosylhomocysteine (AdoHcy) when incubated in Met Hcy⁺ medium. Methionine-independent human tumor cells also have very low levels of free Met compared to normal cells but generally have levels of AdoMet and AdoHcy comparable to normal cells in the Met Hcy⁺ medium. All tumor cell types incorporate amounts of Met into protein similar to normal methionine-independent human fibroblasts when incubated in Met Hcy⁺ medium, thereby indicating apparently normal levels of Met synthesis in the tumor cells. The methionine-independent tumor cell lines in Met Hcy⁺ medium seem able to regulate their AdoMet/AdoHcy ratios normally despite this defect in having very low levels of free Met. Thus, in a diverse set of human tumor cell lines, all are defective in at least one aspect of Met metabolism, giving rise to the possibility of a general metabolic defect in cancer.

Stern, P. H., Wallace, C. D. and *Hoffman, R. M.*

Journal of Cellular Physiology 119:29-34, 1984.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla

ALTERED METHIONINE METABOLISM, DNA METHYLATION AND ONCOGENE EXPRESSION

This well-documented review focuses on the interrelationship of methionine metabolism, DNA methylation and oncogenic transformation. Sections of this study are devoted to: General methionine metabolism; Altered methionine metabolism in cancer — methionine dependence; Effectors of S-adenosylmethionine (AdoMet) metabolism; Altered DNA methylation in cancer; Gene induction due to altered DNA methylation and/or altered methionine metabolism; Effects of methylation on oncogene expression; Chemoprotective effect of methionine against cancer; and Conclusions and perspectives. In the concluding section, a model of one pathway to carcinogenesis is presented. A corresponding figure depicts the related pathways of methionine and methylation metabolism and indicates points in the biochemical scheme where interruptions may lead to carcinogenesis. Six perturbations in the scheme that may affect oncogenesis and the possible mechanisms involved are: (1) Carcinogens may bind guanine residues, prevent methylation of adjacent cytosines and thereby possibly allow oncogene activity. (2) Carcinogens that are reagents for sulfhydryl groups can act directly on DNA methyltransferases and inactivate them. (3) Excess S-adenosylhomo-

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cysteine, such as that found in methionine-dependent cells, can competitively inhibit DNA methyltransferases. (4) The carcinogen ethionine can serve as a substrate for AdoMet synthetase with the production of S-adenosylethionine which in turn can inhibit methylation. (5) If methionine is in short supply, low levels of AdoMet and reduced activity of DNA methyltransferase may result, and (6) Interference in general methionine/methylation metabolism could also interfere with chromosomal proteins. Finally, two important implications of this model as it is presented here are: in many cases oncogenic transformation may be prevented by a diet containing excess methionine, possibly obtained through choline, and if demethylation of DNA is an important carcinogenic event, the possibility arises of remethylating DNA and reversing oncogenesis.

Hoffman, R. M.

Biochimica et Biophysica Acta 738:49-87, 1984.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla.

HYPOMETHYLATION OF DNA HUMAN CANCER CELLS: A SITE-SPECIFIC CHANGE IN THE C-*MYC* ONCOGENE

Site-specific methylation of the human c-*myc* oncogene was investigated in a set of cultured human tumor cell lines and normal human fibroblast strains. As previsouly reported, all the tumor cell lines, in contrast to normal cells, were hypomethylated to various degrees in their total genomic DNA. The presence of methylation in specific regions of the c-*myc* gene was analyzed by use of the restriction endonuclease isoschizomers *Mspl* and *Hpall*, which recognize the sequence 5′-CCGG-3′ (CCGG = DNA sequence of 2 cytosine bases followed by 2 guanine bases) but differ in their abilities to cleave at the internal cytosine residue when it is methylated. The first exon, first intervening sequence and second exon were hypomethylated in all cell types, regardless of whether the cells were normal or oncogenically transformed and regardless of the degree of total genomic methylation of the cell. However, the solitary CCGG site in the third exon was fully methylated in normal cell strains. In contrast, in 3 of 5 tumor cell lines measured, this site was hypomethylated. This is the first demonstration of a site-specific DNA methylation defect in a cellular oncogene. Some possible implications relating disruption of DNA methylation to oncogene control and oncogenesis are discussed.

Cheah, M. S. C., Wallace, C. D., and *Hoffman, R. M.*

Journal of the National Cancer Institute 73(5): 1057-1065, 1984.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, La Jolla, CA.

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ia, San Diego, La Jolla, CA.

ELEVATED OVERALL RATES OF TRANSMETHYLATION IN CELL LINES FROM DIVERSE HUMAN TUMORS

In a study of a diverse set of human tumor cell lines previously shown to have a defect in methionine metabolism, it was demonstrated that all have enhanced overall rates of transmethylation compared to normal human fibroblasts. Transmethylation rates were measured by blocking S-adenosylhomocysteine (AdoHcy) hydrolase and measuring the AdoHcy which accumulates as a result of transmethylation. The enhanced transmethylation rates may be the basis of the above-mentioned defects in methionine metabolism previously reported in human tumor cells, including the inability of the majority of the tumor cells to grow when methionine is replaced by homocysteine. The excess and unbalanced tRNA methylation observed for the last 25 years in many types of cancer may be at least in part explained by our results of elevated rates of overall transmethylation in cancer cells. The alteration of such a fundamental process as transmethylation in cancer may be indicative of its importance in the oncogenic process.

Stern, P. H. and *Hoffman, R. M.*

In Vitro 20(8):663-670, 1984.

Other support: National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, La Jolla.

THE CYTOSKELETON OF MURINE LEUKEMIA VIRUS (MuLV)-INFECTED MOUSE FIBROBLASTS AS OBSERVED UNDER VARYING CONDITIONS OF FORMALDEHYDE FIXATION

In the work reported here, mouse fibroblasts chronically infected with Moloney murine leukemia virus (MuLV) were fixed using variable amounts of formaldehyde, then examined by indirect immunofluorescence light microscopy. Several antisera were employed to detect both external and internal antigens associated with the cells, *e.g.*, MuLV gp70, tubulin, vimentin, and actin. Results indicate that the cell membranes could be partially permeabilized to IgG molecules directed against the three cytoskeletal antigens only after 3.7%, but not 1%, formaldehyde treatment. Complete permeabilization was achieved by subsequent acetone treatment of cells after 3.7% formaldehyde fixation. In such cells, normal-appearing cytoskeletal networks of microtubles and intermediate filaments were observed. Stress fibers were also seen; however, they appeared less numerous and thinner than those of uninfected mouse fibroblasts. Further, a significant amount of F-actin fluorescence was localized in granules in the cytoplasm of infected cells. Similar observations were made using JLS-V9 mouse cells chronically infected with 334C virus, another MuLV. These results taken together suggest that subtle differences exist in the organization of actin within MuLV-infected and uninfected mouse fibroblasts.

Sataka, M., Lupo, D. and *Luftig, R. B.*

Cell Motility 3:567-577, 1983.

Other support: National Cancer Institute.

From the Department of Microbiology and Immunology, University of South Carolina, School of Medicine, Columbia.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

GENETIC PREDISPOSITION TO BREAST CANCER

Although familial clustering of breast cancer was first recorded in the Roman medical literature around 100 A.D., its public health magnitude and significance are still problematic as of this day. In the present epidemiological study, it is shown that breast cancer risk factors are closely intertwined with the patient's cultural background, which may contribute to breast cancer aggregations within families. The difficult questions are: (1) does a truly hereditary breast cancer subset exist?; (2) which familial aggregations are hereditary?; and (3) is the hereditary form distinctive from its sporadic counterpart? These queries will be resolved once biomarkers are identified that show high sensitivity and specificity with genotype. The authors provide a review of this subject and will focus on their recent discovery of increased *in vitro* hyperdiploidy in cultured skin fibroblasts from patients with or at risk for hereditary breast cancer. The authors discuss findings from their study of family histories in 225 consecutively ascertained patients with verified breast cancer from the Creighton University School of Medicine Oncology Clinic. Findings consistent with an hereditary breast cancer syndrome were identified in 5% of the patients. Given the 112,000 new cases of breast cancer in the United States in 1982, the authors estimate that with a confidence coefficient of 0.95 between 2,410 and 8,790 of these individuals will manifest hereditary breast cancer. Specific surveillance/management programs should be geared to high-risk members of these families in which cancer yield will be predictable.

Lynch, H. T. et al.

Cancer 53(3): 612-622, 1984.

From the Creighton University School of Medicine and the Institute for Familial Cancer Management and Control, Inc., Omaha; Cornell University Medical College, New York; University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark; and the Department of Otolaryngology, Boys Town Institute, Omaha.

IS CANCER COMMUNICABLE?

The etiology of cancer remains elusive. Nevertheless, when meticulous clinical laboratory-oriented, medical-genetic/epidemiologic studies are performed on large series of patients with specific histologic forms of cancer, subsets emerge which lend support to a variety of etiologic hypotheses. As presented in this report, recent developments in cancer epidemiology have led to the possibility of an exceedingly complex communicable factor(s) in cancer etiology. The transmission of such an agent(s) may require a susceptible genotype and/or other promotional events. Likely candidates which support this supposition include: (1) Epstein-Barr virus (nasopharyngeal carcinoma, Burkitt's lymphoma, salivary gland tumor among Eskimos, X-linked lymphoproliferative syndrome of Purtilo); (2) human T-cell leukemia virus (adult T-cell leukemia); (3) acquired immune deficiency syndrome (AIDS), complicated by Kaposi's sarcoma (etiologic agent remains elusive, though epidemiology suggests possible infectious transmission); (4) abnormal immune phenomena in households of Hodgkin's disease patients; and (5) clustering of various types of cancer in spouses, the general population, and families. The authors have selectively reviewed the literature and evolved an etiologic hypothesis that integrates a communicable agent(s) in concert with genetic and/or environmental carcinogenic interaction which could conceivably explain a significant fraction of the total cancer burden.

Lynch, H. T., Schuelke,

Medical Hypotheses 14:1

From Creighton Univers
Medicine/Public Health

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Lynch, H. T. et al.

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Lynch, H. T., Schuelke, G. S. and O'Hara, M. K.

Medical Hypotheses 14:181-198, 1984.

From Creighton University School of Medicine, Departments of Preventive Medicine/Public Health and Medical Microbiology, Omaha.

GENETICS, BIOMARKERS, AND CONTROL OF BREAST CANCER: A REVIEW

Though there has been much interest throughout the years in the epidemiology of breast cancer, only very little attention has been given to the role of genetics in its etiology. The review presented here, however, represents an attempt by the investigators to provide a comprehensive coverage of hereditary breast cancer. Included are pertinent endogeneous and exogeneous risk factors which in certain circumstances may significantly influence the role of primary genetic factors. Hereditary breast cancer is heterogeneous. When discussing the subject, therefore, one must be precise relevant to the particular heterogeneous form of concern, based on differing tumor associations. It is probably not appropriate to discuss "hereditary breast cancer" without qualification of the specific hereditary cancer syndrome of concern, *i.e.*, the SBLA syndrome, breast/ovarian cancer syndrome, and others. This reasoning also applied to attempts at linking biomarkers to hereditary breast cancer. Finally, in addition to ongoing discussions on the cardinal principles that associate with hereditary forms of breast cancer, its frequency and new developments in biomarkers, the investigators have provided surveillance/management programs that embrace those facets of the natural history of this disease.

Lynch, H. T., et al.

Cancer Genetics and Cytogenetics 13:43-92, 1984.

Other support: Fraternal Order of Eagles.

From the Department of Preventive Medicine/Public Health, Department of Surgery, Hereditary Cancer Institute, Creighton University School of Medicine, Omaha; the Department of Clinical Pathology, University of Medicine and Dentistry of New Jersey, New Jersey College of Medicine, Newark; and the Department of Medicine, Laboratory for Cell Biology, Cornell University Medical College, New York.

TUMOR INVASION AND HOST EXTRACELLULAR MATRIX

This review discusses some of the major mechanistic pathways by which tumor cells are thought to invade host tissues. Tumor invasion has been conceived to be the result of pathological, close-range interactions between malignant cells and host stroma. The sequence of events that characterize invasion can be summarized as follows: (a) Tumor cell clusters break from the confinement of the primary tumor. Loss of intercellular junctions (desmosomes, gap junctions), alterations in the chemical composition and physical properties of the cell surface coat (loss of fibronectin and heparan sulfate; excess amounts of hyaluronate), and loosening of cell-substrate interactions (loss of hemidesmosomes, fibronectin and heparan sulfate), are among the most frequently listed causes of tumor cell shedding. (b) Increased proteolytic activities at the invasion front cause focal alterations in the surrounding extracellular matrix,

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thereby changing its physical properties. Collagenases and cathepsins as well as elastase and other neutral proteinases are the enzymes most frequently associated with matrix destruction and invasion. In some tissues this process is effectively regulated by inhibitors of matrix-degrading, proteolytic enzymes. (c) Tumor cells migrate into the altered matrix, possibly moving as aggregates along guidance tracks provided by host structures (blood vessels, lymphatics, nerves) or matrix macromolecules (collagen and fibronectin tracks). Migration seems to be preceded by increased swelling of glycosaminoglycan (i.e., hyaluronate) in the matrix, ahead of the migrating cell population. Various host cell types (mast cells, fibroblasts, endothelial cells, macrophages, etc.) may participate in these events.

Pauli, B. U., Schwartz, D. E., Thonar, E. J.-M., and *Kuettner, K. E.*

Cancer Metastasis Reviews 2:129-152, 1983.

Other support: National Cancer Institute.

From the Departments of Pathology, Biochemistry, and Orthopedic Surgery, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

SIMULTANEOUS FEULGEN DENSITOMETRY AND AUTORADIOGRAPHIC GRAIN COUNTING WITH THE QUANTIMET 720D IMAGE-ANALYSIS SYSTEM: III. IMPROVEMENTS IN FEULGEN DENSITOMETRY

A method has been developed for estimation of the Feulgen-stained DNA content of ^{3}H-thymidine-labeled cells in autoradiographs in conjunction with automated grain count measurements using a Quantimet television imaging system. Refinements in the methodology are reported which include (1) the incorporation of an Image-Editor Module into the Quantimet module configuration; (2) the optimization of incident illumination based upon evaluation of various light sources; (3) changes in the optical configuration which reduce glare and minimize the level of monitor shading correction; (4) the optimization of scanner sensitivity; and (5) the evaluation of cell-flattening and staining with respect to densitometry resolution and sensitivity. These refinements resulted in a coefficient of variation (CV) of less than 6.4% in the G-1 and G-2 DNA peaks of rat kidney cells in autoradiographs compared to the previous CV of 10.5%, and a G-2 to G-1 ratio of 2.025. For a fixed field position the CV was 5.1% and the replication error less than 1.0%. This technology is of interest because of its potential application in the cell cycle analysis of mixed polyploid tumor subpopulations.

Sklarew, R. J.

The Journal of Histochemistry and Cytochemistry 31(10):1224-1232, 1983.

From New York University Research Service, New York University Medical Center, Goldwater Memorial Hospital, Roosevelt Island, New York.

CYTOKINETICS OF HETEROPLOID TUMOR SUBPOPULATIONS BY COMBINED AUTORADIOGRAPHIC IMAGING AND FEULGEN DENSITOMETRY

In human tumors where polyploidy and aneuploid variants frequently coexist, the traditional autoradiographic methods for analysis of the cell cycle kinetics of tumors, which have been limited to estimation of mean phase transit-times and their dispersion

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Sklarew, R. J.

In: Proceedings of the 10t
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in the population as a whole, do not relate to the cytokinetic behavior of the distinct ploidy lineages. In this study, an approach is described for achieving a deconvolution of the cell cycle phase distribution in mixed heteroploid tumor populations during exponential growth. The scheme provides (1) the population frequency and phase distribution of discrete subpopulations distinguished by their DNA ploidy level; (2) their transit-time kinetics as revealed by serial estimates of compartmental turnover in S, G2, G1 and in the overall cycle; and (3) their cell flux contributions at the S/G2, G2/Mitosis and G1/S phase boundaries. The analysis is facilitated by an automated methodology developed with a Quantimet 720D image-analysis system for simultaneous determination of the DNA content and grain count of ^{3}H-TdR labeled cells in Feulgen-stained autographs. The designs involve ^{3}H- and ^{14}C-TdR pulse-labeling, double-labeling and continuous labeling in conjunction with Colcemid. The procedure has been computerized from acquisition of imaging data, through the calculation of relevant parameters and their graphic display. The analysis is illustrated in a heteroploid, uncloned MCF-7 culture subline. This approach circumvents the intractable problem of resolving mixed heteroploid and aneuploid subpopulations from their composite DNA distribution where G1, S and G2 components at various levels overlap extensively in their DNA content range. In the future, it seems reasonable that the use of ploidy markers to identify clonal lineages (subpopulations) may ultimately provide a basis for determining their sensitivity and resistance to a variety of agents in relation to their cycle phase distribution and overall frequency.

Sklarew, R. J.

In: Proceedings of the 10th International Cell Cycle Conference, Banff Centre, Alberta, Canada, April 24-28, 1984. The Humana Press Inc., Clifton, NJ.

From New York University School of Medicine, Goldwater Memorial Hospital, Roosevelt Island, New York.

CYTOKINETICS OF SUBPOPULATIONS IN MIXED HETEROPLOID TUMORS BY TELEVISION IMAGING. I. DECONVOLUTION OF THE S-PHASE DNA PLOIDY COMPOSITION. II. ANALYSIS OF THE S-PHASE EMPTYING PROFILE OF PLOIDY SUBPOPULATIONS

The problem of evaluating the cell cycle kinetics of discrete subpopulations in mixed tumor cell populations has been most intractable. However, a scheme has been developed for deciphering the cell cycle time parameters of cell subpopulations that differ in their DNA ploidy level and that coexist in mixed heteroploid tumors. In this paper, the S-phase analysis is presented. The approach is coupled to an automated imaging methodology for simultaneous determination of the Feulgen-stained DNA content and grain count of ^{3}H-thymidine-labeled cells in autoradiographs (Sklarew RJ: J Histochem Cytochem 30:35, 30:49, 1982). The experimental designs involve ^{3}H- and ^{14}C-thymidine double labeling and Colcemid incubation. The deconvolution of the S-phase ploidy composition is illustrated in rat sarcoma cultures comprising four major ploidy subpopulations with G-2 and mitotic DNA contents of approximately 4C, 8C, 16C, and 32C. The components were identified by their DNA ploidy level, and their S frequencies and labeling indices were obtained. A scheme is also developed and validated for obtaining the S-phase emptying profile of component ploidy subpopulations, and their cell flux at the S/G-2 and G-2/mitosis phase boundaries. In the

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sarcoma cultures S mobility was found to decrease with increasing DNA ploidy level over the entire ploidy range.

Sklarew, R. J.

The Journal of Histochemistry and Cytochemistry 32(4):413-420, 1984.

From the Department of Pathology, New York University Research Service, New York University School of Medicine, Goldwater Memorial Hospital, Roosevelt Island, New York.

CYTOKINETICS OF SUBPOPULATIONS IN MIXED POLYPLOID TUMORS BY TELEVISION IMAGING. III. ^{3}H-THYMIDINE INCORPORATION BY PLOIDY SUBPOPULATIONS—CONTROL OF ^{3}H-ABSORPTION AND EMULSION EFFICIENCY IN AUTORADIOGRAPHY

In this cell-culture study, an approach is presented for determining relative rates of ^{3}H-thymidine incorporation in S-phase for discrete ploidy subpopulations that coexist in heteroploid tumors. The analysis is facilitated by an automated imaging methodology developed with a Quantimet 720 System for simultaneous determination of Feulgen-stained DNA content and grain counts of ^{3}H-thymidine-labeled cells in Feulgen-stained autoradiographs. The experimental design involves ^{3}H- and ^{14}C-thymidine double labeling in the presence of Colcemid. Data from heteroploid rat sarcoma cultures illustrate the profound influence of differential ^{3}H absorption and relative emulsion efficiency. These factors are controlled, respectively, through measurements of mean nuclear optical density and grain density. This is essential for a valid comparison of grain counts between cells that differ in their autoradiographic geometry. Thus, relative DNA specific activity may be assessed in individual cells. The protocol and analysis support a simultaneous evaluation of the S-phase ploidy composition and S-emptying profile, as presented in a companion article abstracted here.

Sklarew, R. J.

The Journal of Histochemistry and Cytochemistry 32(4):421,-431,1984.

From the Department of Pathology, New York University Research Service, New York University School of Medicine, Goldwater Memorial Hospital, Roosevelt Island, New York.

PLASMID, PHAGE, AND GENOMIC DNA-MEDIATED TRANSFER AND EXPRESSION OF PROKARYOTIC AND EUKARYOTIC GENES IN CULTURED HUMAN CELLS

Transfection of mammalian cells with genomic DNA and cloned genes is now relatively routine. However, the vast majority of studies have used rodent cells as recipients. Here the authors describe efficient transfection of two human cell lines, the hypoxanthine guanine phosphoribosyltransferase (HPRT)-deficient HeLa line, D98/AH-2, and the adenine phosphoribosyltransferase (APRT)-deficient HT1080 line, HTD114. D98/AH-2 cells were transfected with the pSV2-gpt plasmid of Mulligan and Berg, which contains the *E. coli* xanthineguanine phosphoribosyltransferase (gpt) gene, and Gpt$^+$ transfectants were selected in HAT medium. HTD114 cells were transfected with: (1) genomic hamster DNA, and ouabain resistant transfectants were selected in 5 x 10^{-3} M ouabain; (2) hamster and mouse genomic DNA, and Aprt$^+$ cells

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Benedict, W. F., Wei

Cancer Research 44:3

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were selected in AAA medium; (3) plasmids containing either the cloned hamster or mouse APRT genes, and Aprt$^+$ cells were selected; and (4) phage particles containing a cloned mouse APRT gene, and Aprt$^+$ cells were selected. Transfection efficiencies ranged from 0.25 to 1.5 x 10^3 transfectants per μg DNA, and in certain cases secondary transfections were done. Foreign DNA in recipients was detected by blot hybridization, and the expression of foreign genes was detected by cell growth in selective media and the expression of enzymes characteristic of the species of the donor DNA. The majority of transfectants showed stable expression of the transgenome.

Srivatsan, E. S., *Stanbridge, E. J.*, Saxon, P. J., Stambrook, P. J., Trill, J. J., and Tischfield, J. A.

Cytogenetics and Cell Genetics 38: 227-234, 1984.

Other support: National Institutes of Health and the National Science Foundation.

From the Department of Microbiology, College of Medicine, University of California, Irvine; Department of Anatomy and Cell Biology, College of Medicine, University of Cincinnati, OH; and Department of Anatomy, Medical College of Georgia, Augusta.

TUMORIGENICITY OF HUMAN HT1080 FIBROSARCOMA X NORMAL FIBROBLAST HYBRIDS: CHROMOSOME DOSAGE DEPENDENCY

The tumorigenic capacity of hybrids formed by fusion of the highly tumorigenic HT1080 human fibrosarcoma cell line with nontumorigenic normal fibroblasts was examined. The HT1080 also contains an activated N-ras oncogene. Near-tetraploid hybrids which contained an approximately complete chromosomal complement from both parental cells were nontumorigenic when 1×10^7 cells were injected s.c. into athymic (nude) mice, whereas the parental HT1080 cells produced tumors in 100% of the animals with no latency period following injection of 2×10^6 cells. Tumorigenic variants were obtained from these hybrids which had lost only a few chromosomes compared to cells from the nontumorigenic mass cultures. In addition, several near-hexaploid hybrids were obtained which contained approximately a double chromosomal complement from the HT1080 parental line and a single chromosomal complement from the normal fibroblasts. All of these near-hexaploid hybrids produced tumors in 100% of nude mice with no latency period. Results of this study indicate that tumorigenicity of these particular human malignant cells of mesenchymal origin can be suppressed when fused with normal diploid fibroblasts. In addition, the results suggest that tumorigenicity in this system is chromosomal dosage dependent, since a diploid chromosomal complement from normal fibroblasts is capable of suppressing the tumorigenicity of a near-diploid but not a near-tetraploid chromosomal complement from the tumorigenic HT1080 parent. Finally, the loss of chromosome 1 (the chromosome to which the N-ras oncogene has been assigned) as well as chromosome 4 was correlated with the reappearance of tumorigenicity in the rare variant populations from otherwise nontumorigenic near-tetraploid hybrid cultures. These results also suggest the possibility that tumorigenicity in these hybrids may be a gene dosage effect involving the number of activated N-ras genes in the hybrids compared to the gene(s) controlling the suppression of the activated N-ras genes.

Benedict, W. F., Weissman, B. E., Mark, C., and *Stanbridge, E. J.*

Cancer Research 44:3471-3479, 1984.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Other support: National Cancer Institute.

From the Division of Hematology-Oncology, Childrens Hospital of Los Angeles; Department of Microbiology, College of Medicine, University of California, Irvine; and Department of Pediatrics, University of Southern California, School of Medicine, Los Angeles.

II. *The Respiratory System*

NICOTINE IS CHEMOTACTIC FOR NEUTROPHILS AND ENHANCES NEUTROPHIL RESPONSIVENESS TO CHEMOTACTIC PEPTIDES

On the basis of this *in vitro* study, it is reported that nicotine, which is readily absorbed from the respiratory tract, has effects on polymorphonuclear neutrophils that may be relevant to lung inflammation *in vivo*. Nicotine was found to be chemotactic for human neutrophils but not monocytes, with a peak activity at ~ 31 micromolar. In lower concentrations (comparable to those in smokers' plasma), nicotine enhanced the response of neutrophils to two chemotactic peptides. In contrast to most other chemoattractants for neutrophils, however, nicotine did not affect degranulation or superoxide production. Nicotine thus may promote inflammation and consequent lung injury in smokers.

Totti, N. III, McCusker, K. T., *Campbell, E. J.*, Griffin, G. L., and Senior, R. M. *Science* 223:169-171, 1984.

Other support: U. S. Public Health Service.

From the Respiratory and Critical Care Division, Department of Medicine, Jewish Hospital of St. Louis, Washington University Medical Center, St. Louis.

NEUTRAL PROTEINASES FROM HUMAN INFLAMMATORY CELLS: A CRITICAL REVIEW OF THEIR ROLE IN EXTRACELLULAR MATRIX DEGRADATION

In inflammatory reactions, proteinases and a variety of other leukocytic products are released into the extracellular environment. The proteolytic enzymes released by inflammatory cells initiate the digestion of nonviable tissue, fibrin, and denatured proteins and proteoglycans, and may activate mediators of inflammation. In the review presented here, three things are considered: (1) the cell biology and *in vitro* activity of neutral proteinases derived from human inflammatory cells, (2) mechanisms by which these enzymes might injure connective tissue *in vivo*, and (3) the biologic activity of peptides released from connective tissue by proteinases. To summarize this paper, human inflammatory cells, particularly neutrophils, release neutral proteinases that have the capacity to degrade extra-cellular matrix molecules. Neutrophil elastase is especially notable because it is present in large amounts and has the capacity to degrade elastin and many other components, including basement membrane collagen (type IV), proteoglycans and fibr
suggesting an important role
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Senior, R. M. and *Campbe*

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Other support: U. S. Publ
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From the Department of Me
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PATHOGENESIS OF THI EVIDENCE OF OXIDAN FLUID

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Cochrane, C. G., Spragg,

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Other support: National In

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

IV), proteoglycans and fibronectin. Although there is much circumstantial evidence suggesting an important role for neutrophil-derived neutral proteinases in extracellular matrix breakdown in inflammatory reactions, direct quantification of connective tissue proteolysis by neutrophils in human disease has not yet been possible. Tissue destruction in association with neutrophilic infiltration is clearly a complex process that may involve proteinases, oxidants, proteinase inhibitors, and other substances from resident cells as well as neutrophil products. In addition, compared with neutrophils, human mononuclear phagocytes release very small amounts of neutral proteinase activity and also synthesize proteinase inhibitors, so the role of these cells in the proteolysis of tissues is even more obscure than the role of neutrophils.

Senior, R. M. and *Campbell, E. J.*

Clinics in Laboratory Medicine 3(4):645-666, 1983.

Other support: U. S. Public Health Service and the American Lung Association of Eastern Missouri.

From the Department of Medicine, Washington University School of Medicine at The Jewish Hospital of St. Louis, St. Louis.

PATHOGENESIS OF THE ADULT RESPIRATORY DISTRESS SYNDROME: EVIDENCE OF OXIDANT ACTIVITY IN BRONCHOALVEOLAR LAVAGE FLUID

The present study provides evidence indicating that oxidants are generated in lungs of patients with the adult respiratory distress syndrome (ARDS). The evidence was derived from observations that α-1-PI (alpha-1-proteinase inhibitor), recovered in bronchoalveolar lavage (BAL) fluid, had been inactivated by oxidation, presumably oxidation of the methionyl residue in the reaction site of the molecule. This was indicated by findings that activity of the α-1-PI could be restored by exposure to the reducing agent, dithiothreitol in the presence of methionyl sulfoxide peptide reductase. The amount of activity restored was proportional to the amount of inactive α-1-PI present at 52,000 D. Oxidation of the 52,000-D α-1-PI was also revealed by the finding that the inactive molecule was subject to proteolytic cleavage to 47,000 D when exposed to porcine pancreatic elastase. Inactivation of the α-1-PI *in vivo* also resulted from complexing to an active enzyme, shown previously to be neutrophil elastase. In contrast to that in BAL fluids, the α-1-PI in plasma of patients with respiratory distress syndrome was found to be >90% active in 14 of 22 cases and 50-90% active in 8 cases. This suggested that for the most part, α-1-PI was inactivated after leaving the vessels and entering the lung. The circulating α-1-PI in patients with the respiratory distress syndrome was found to be equally susceptible to oxidative inactivation as α-1-PI from normal individuals. It seems improbable therefore that patients develop ARDS because of labile α-1-PI inhibitor.

Cochrane, C. G., Spragg, R. and Revak, S. D.

Journal of Clinical Investigation 71:754-761, 1983.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA, and the Pulmonary Division, University of California, San Diego, La Jolla

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

PROTEASES AND OXIDANTS IN EXPERIMENTAL PULMONARY INFLAMMATORY INJURY

The investigators have exmined various biochemical parameters of pulmonary inflammation in experimental animals. Intrabronchial instillation of glucose oxidase-glucose (GO/G) to produce oxidants or formylated norleu-leu-phe (FNLP) or phorbol myristate acetate (PMA) as leukocytic stimuli induced severe acute pulmonary injury in New Zealand white rabbits. PMA also induced inflammation when administered intravenously. Each stimulus induced transudation of protein from the vascular space into the pulmonary tissues, and an influx of leukocytes during the 4-6 h period of the experiment. Pathophysiologic changes were measured by edema formation (transudation of ^{125}I-bovine serum albumin), and histologic examination. Biochemical analysis was performed by measuring concentrations of potentially injurious agents in bronchoalveolar lavage (BAL) fluid. Increased acid protease and myeloperoxidase levels were found in the BAL fluid after administration of either of the stimuli.

Schraufstätter, I. U., Revak, S. D., and *Cochrane, G. G.*

Journal of Clinical Investigation 73:1175-1184, 1984.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA.

BIOCHEMICAL FACTORS IN PULMONARY INFLAMMATORY DISEASE

Various biochemical events taking place during pulmonary inflammation were examined in the bronchoalveolar lavage (BAL) fluids from patients with acute respiratory distress syndrome (ARDS) and in experimental animal models. In patients with ARDS, active neutrophil elastase was found in the BAL fluids. In these fluids, inactivation of the major elastase inhibitor α_1-protease inhibitor (α_1-PI) occurred. This was caused by oxidation of a methionine residue at the active site of the α_1-PI, and offered indirect evidence of oxidation occurring in the inflamed pulmonary tissues. Studies with experimental animals have been initiated to gain understanding of the relative roles of proteases, oxidants, arachidonate metabolites, complement and contact system components, and other mediators in the pathogenesis of pulmonary inflammation. Intrabronchial instillation of glucose oxidase/glucose to produce oxidants or formylated norleucylleucylphenylalanine or phorbol myristate acetate as leukocytic stimuli induced severe acute pulmonary injury in New Zealand white rabbits and rhesus monkeys. The injury was accompanied by leukocytic protease (acid cathepsins) release in rabbit lungs and oxidant formation, and could be inhibited by neutrophil depletion. Oxidant formation was demonstrated by the inactivation of catalase by 3-amino-1,2,4-triazole in the presence of H_2O_2, a drop in intracellular glutathione levels, and in the rhesus monkey by inactivation of α_1-PI.

Schraufstätter, I., Revak, S. D., and *Cochrane, C. G.*

Federation Proceedings 43:2807-2810, 1984.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA.

CERULOPLASMIN: INCRI
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Galdston, M. et al.

American Review of Respir

Other support: The Louis

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CERULOPLASMIN: INCREASED SERUM CONCENTRATION AND IMPAIRED ANTIOXIDANT ACTIVITY IN CIGARETTE SMOKERS, AND ABILITY TO PREVENT SUPPRESSION OF ELASTASE INHIBITORY CAPACITY OF ALPHA$_1$-PROTEINASE INHIBITOR

Bronchoalveolar lavage fluid of smokers and nonsmokers contains significant concentrations of ceruloplasmin, the major serum inhibitor of lipid peroxidation, with limited superoxide dismutase activity. This suggested that ceruloplasmin may protect the lower respiratory tract against oxidant(s) in cigarette smoke and air pollutants. To follow up on this, these investigators studied (1) serum ceruloplasmin concentration and antioxidant activity (percentage inhibition of autoxidation of ox-brain homogenate) in healthy male and female smokers and nonsmokers, and (2) the capacity of ceruloplasmin to prevent suppression of the elastase inhibitory capacity of alpha$_1$-proteinase inhibitor by the oxidant chloramine T and by cigarette smoke solution. Mean ceruloplasmin concentration was significantly higher in smokers than in nonsmokers and in females than in males whether or not they smoked. Serum antioxidant activity showed significant linear correlations with serum cerulaplasmin in smokers and nonsmokers of both sexes. There was a linear relationship between ceruloplasmin concentration and its ability to prevent suppression of the elastase inhibitory capacity of alpha$_1$-proteinase inhibitor by chloramine T and cigarette smoke solution. These findings indicate (1) that cigarette smoking can cause partial inactivation of serum antioxidant activity accompanied by insufficient compensatory increase in ceruloplasmin concentration, and (2) that ceruloplasmin may protect the lung against oxidant(s) in cigarette smoke and air pollutants.

Galdston, M. et al.

American Review of Respiratory Disease 129:258-263, 1984.

Other support: The Louis and Rose Klosk Fund.

From the Department of Medicine and the Chest Service, Bellevue Hospital, and the Department of Environmental Medicine, New York University Medical Center, New York.

CIGARETTE SMOKE-INDUCED BRONCHOCONSTRICTION IN DOGS: VAGAL AND EXTRAVAGAL MECHANISMS

The authors of this paper studied the mechanism of cigarette smoke-induced bronchoconstriction by methods that allowed separation of vagal afferent and efferent routes. To evaluate the severity of smoke-induced bronchoconstriction in anesthetized dogs, they measured airway pressure and airflow resistance (Rrs, forced oscillation method). They studied the mechanisms in other dogs by measuring airway pressure, central airway smooth muscle tone in tracheal segments *in situ*, and respiratory center drive by monitoring phrenic motor nerve output, including the role of vagal and extravagal nerves vs. the role of blood-borne materials during inhalation of cigarette smoke. Rrs increased more than fourfold with smoke from one cigarette delivered in two tidal volumes. About half the airway response was due to local effects of smoke in the lungs. The remainder was due to stimulation of the respiratory center, which activated vagal motor efferents to the airway smooth muscle. Of this central stimulation, about half was due to blood-borne materials and the rest to vagal pulmonary afferents from the lungs. These investigators conclude that inhalation of cigarette

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

smoke in dogs causes severe bronchoconstriction which is mediated mainly by extravagal mechanisms.

Hartiala, J., Mapp, C., Mitchell, R. A., Shields, R. L., and *Gold, W. M.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 57(4):1261-1270, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Cardiovascular Research Institute, Departments of Medicine and Physiology, University of California, San Fransisco.

LOCALIZATION OF CALMODULIN IN DIFFERENTIATING PULMONARY TYPE II EPITHELIAL CELLS

Pulmonary surfactant, a complex mixture of lipid, protein, and carbohydrate which lines alveolar surfaces, is synthesized by alveolar type II pneumocytes and stored in inclusions called lamellar bodies. In the present study the researchers have investigated the role of the calcium-binding protein, calmodulin, in regulating surfactant secretion in differentiating rat fetal type II pneumocytes. Lamellar body secretion is stimulated in differentiating type II cells *in vitro* by the calcium ionophore, A23187. A23187-induced secretion is blocked by the phenothiazine drugs, trifluoperazine and chlorpromazine, but is unaffected by the inactive analogs, trifluoperazine sulfoxide and chlorpromazine sulfoxide. Immunofluorescence studies on cultured type II pneumocytes show that the percentage of Nomarski-dense intracellular granules, which stain positively with anticalmodulin antibody, increases when the cells are stimulated with the calcium ionophore, A23187. Since these Nomarski-dense granules are positively stained by phosphine-3R, these results indicate that increased amounts of immunoreactive calmodulin appear associated with lamellar body surfaces when the cells are stimulated for secretion. In addition, ultrastructual localization of calmodulin on isolated lamellar bodies using protein A-colloidal gold indicates that calmodulin is present on their outer surfaces. Taken together, these and other results implicate calmodulin in pulmonary surfactant secretion.

Hill, D. J., Wright, T. C., Jr., Andrews, M. L., and *Karnovsky, M. J.*

Laboratory Investigation 51(3):297-306, 1984.

Other support: National Cancer Institute.

From the Department of Pathology, Harvard Medical School, Boston.

SEROTONIN AND THE PULMONARY CIRCULATION

Although serotonin (5-hydroxytryptamine, 5-HT) has been discredited as the mediator of hypoxic pulmonary vasoconstriction, it continues to be considered one of the vasoconstricting substances and has been demonstrated in the lungs of all species investigated. In summarizing earlier studies, evidence has been offered that removal of 5-HT is inhibited by hypothermia or hyperoxia, is not influenced by hypoxia, is inhibited by anoxia (which should be considered separately from physiologic hypoxia), and is also inhibited by cyanide, imipramine, chlorpromazine, and ouabain, among others. These kinetic and inhibitor studies support the criteria for active trans-

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Will, J. A., *Keith, I. M.*, B
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Saria, A., *Lundberg, J.*

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port. The work reported in this chapter originates from earlier studies of pharmacologic modification of pulmonary arterial function and morphology. As part of an investigation of the effects of selected systemic vasodilators on the pulmonary circulation, ketanserin (R41468) and *dl*-p-chlorophenylalanine (*p*-CPA) were tested. A third study was initiated to confirm or reject the results of the earlier ketanserin study and contrast its activity with another 5-HT_2 inhibitor, cyproheptadine (Periactin). In summary, these studies support the previously reported suggestion that the pathogenetic mechanism of RV hypertrophy and medial thickening in chronic hypoxia may not be the same. Furthermore, serotonin seems to exhibit both excitatory and inhibitory activities on pulmonary arteries of 50 to 100 μM in size. This effect was seen by either depleting 5-HT with *p*-CPA treatment or by blocking the 5-HT_2 receptors with ketanserin. These experiments focuses attention on new concepts and possibilities for the control of the pulmonary circulation and is compatible with the hypothesis that normoxic pulmonary vasodilation is the active phase of the pulmonary vascular response to hypoxia.

Will, J. A., *Keith, I. M.*, Buckner, C. K., Chacko, J., Olson, E. B., Jr., and Weir, E. K.

In: Becker, K. L. and Gazdar, A. F. (eds.): *The Endocrine Lung in Health & Disease*, Philadelphia: W. B. Saunders, 1984, pp. 137-154.

Other support: College of Agricultural and Life Sciences, Air Force, and the National Institutes of Health.

From the Department of Veterinary Science, University of Wisconsin, Madison.

VASCULAR PROTEIN LEAKAGE IN VARIOUS TISSUES INDUCED BY SUBSTANCE P, CAPSAICIN, BRADYKININ, SEROTONIN, HISTAMINE AND BY ANTIGEN CHALLENGE

In the work reported here: (1) Plasma extravasation was induced in rats or guinea pigs by intravenous injections of 1. substance P (SP), 2. the C-terminal SP-hexapeptide SP(6-11), 3. serotonin (5-HT), 4. histamine, 5. bradykinin, 6. capsaicin and 7. by antigen challenge. (2) Plasma extravasation induced by SP, SP(6-11), by 5-HT and by capsaicin was, with few exceptions, observed in the same tissues. The effect of SP was not blocked by H_1 and H_2 histamine receptor antagonists. The effect of i.v. capsaicin was absent in capsaicin desensitized animals. Plasma extravasation upon i.v. SP, SP(6-11), 5-HT and capsaicin was seen in the skin and in all organs containing mucus membranes except the intestinal mucosa, and (3) Plasma extravasation by histamine, bradykinin, and antigen challenge of sensitized guinea pigs was also observed in the stomach and intestine. Plasma extravasation and bronchoconstriction by antigen challenge with 20 μg/kg ovalbumin was completely blocked by combined H_1 and H_2 histamine receptor blockade. Both responses were reduced to about the half capsaicin desensitized guinea pigs, though the reduction of the permeability response was statistically not significant in all organs. In conclusion, several substances including anaphylaxis induce protein leakage in many tissues with differing selective distribution patterns. Anaphylactic histamine release leads to protein leakage partly via activation of sensory neurons. SP is a likely mediator of neurogenic protein leakage in many organs.

Saria, A., *Lundberg, J. M.*, Skofitsch, G., and Lembeck, F.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Naunyn-Schmiedeberg's Archives of Pharmacology 324:212-218, 1983.

Other support: Austrian Scientific Research Fund, Swedish Medical Research Council, Swedish Tobacco Company, Wilbergs Stiftelse, Hans och Loo Ostermans Stiftelse, Gustav V Foundation, Svenska Läkaresällskapet, Magnus Bergvalls Stiftelse, Augusta and Petrus Hedlungs Stiftelse, and Astra Foundation.

From the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria, and the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

SUBSTANCE P AND CAPSAICIN-INDUCED CONTRACTION OF HUMAN BRONCHI

In the present study the effects of substance P (SP) and capsaicin on human bronchial smooth muscle tone were monitored *in vitro*. Results showed that SP induced a dose-dependent contraction of human segmental bronchi *in vitro* with a threshold dose of about 10^{-9} M. These preparations were obtained from patients undergoing lung tumor surgery. The SP-induced contractions were resistant to mepyramine and atropine, suggesting a direct effect on the bronchial smooth muscle. Capsaicin (10^{-5} M) also induced a slowly developing, strong atropine-resistant contraction of human bronchi *in vitro*. A rapid tachyphylaxis developed for the response to capsaicin. Both SP and capsaicin were less potent than acetylcholine and histamine in inducing contractions of human bronchi. This finding may, however, be partly due to the experimental conditions; both SP and capsaicin were comparatively much more potent in guinea-pig preparations. Transmural field stimulation of the bronchial preparations in man resulted in contractions that were largely sensitive to atropine. The presence of capsaicin-induced bronchial contractions, however, indicates the existence of a local noncholinergic axon-reflex control of bronchial smooth muscle tone by SP in man.

Lundberg, J. M., Martling, C-R. and Saria, A.

Acta Physiologica Scandinavica 119:49-53, 1983.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Wibergs Stiftelse, Hans och Loo Ostermans Stiftelse, Gustaf V Foundation, Karolinska Institutet Fonder, Svenska Läkaresällskapet, Magnus Bergvalls Stiftelse, Austrian Scientific Research Fund, Petrus and Augusta Hedlunds Fund, and Astra Foundation.

From the Department of Pharmacology, Karolinska Institutet, and Department of Anesthesiology, Karolinska Hospital, Stockholm, Sweden; and Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria.

EFFECTS AND DISTRIBUTION OF VAGAL CAPSAICIN-SENSITIVE SUBSTANCE P NEURONS WITH SPECIAL REFERENCE TO THE TRACHEA AND LUNGS

The origin of substance P (SP)-immunoreactive neurons in the lower respiratory tract, esophagus and heart of guinea pigs was demonstrated in some of these studies by surgical denervation or capsaicin pretreatment with subsequent determination of the tissued levels of SP by radioimmunoassay. In other experiments the effect of vagal nerve stimulation on the SP levels in these tissues was studied. The effects of cap-

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Journal of Applied Ph
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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

saicin-sensitive afferents in the respiratory tract mucosa and bronchial smooth muscle was also studied by analysis of vascular permeability to Evans blue and insufflation-pressure changes. The present data indicate that all SP nerves in the trachea and lung are afferent and capsaicin-sensitive. The trachea and stem bronchi receive SP afferents mainly from the right vagus nerve with cell bodies located in both the nodose and jugular ganglia. The SP innervation of the lung seems to have a dual origin: 1. Afferents from both vagal nerves with a crossed type of innervation pattern; 2. A non-vagal source which consists of about 40% of the SP nerves in the lung. These nerves probably originate from thoracic spinal ganglia. The effects of ether and capsaicin on insufflation pressure and increase in vascular permeability were dependent on the integrity of capsaicin-sensitive afferents of both vagal and non-vagal origin. In the guinea pig, systemic capsaicin pretreatment to adult animals seemed to result in irreversible changes in the respiratory tract, while in the rat a successive recovery of the functional response of capsaicin-sensitive afferents occurred.

Lundberg, J. M., Brodin, E. and Saria, A.

Acta Physiologica Scandinavica 119:243-252, 1983.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Wibergs Stiftelse, Hans och Loo Ostermans Stiftelse, Gustaf V Foundation, Astra Foundation, Magnus Bergvalls Stiftelse, Karolinska Institutet Forskningsfonder and Svenska Läkaresällskapet.

From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

EFFECT OF ROUTE OF ATROPINE DELIVERY ON BRONCHOSPASM FROM COLD AIR AND METHACHOLINE

The authors undertook a study to determine whether the apparent disparity between the dose of inhaled atropine required to inhibit bronchoconstriction induced by inhaled methacholine and the dose required to inhibit the bronchoconstriction induced by eucapnic hyperpnea with cold air is a function of the route of administration of atropine. In six subjects with asthma, they constructed dose-response curves to inhaled methacholine and to eucapnic hyperpnea with cold air after treatment with inhaled atropine (0.5 mg delivered) and intravenous placebo, with inhaled placebo and intravenous atropine (0.5 mg injected), and with inhaled and intravenous placebos. Atropine by either route shifted the dose-response curves to both cold air and to methacholine to the right. In every subject, however, inhaled atropine caused a markedly greater rightward shift of the inhaled methacholine dose-response curve than did intravenous atropine, whereas inhaled and intravenous atropine had similar effects on the cold air dose-response curve. These findings suggest that the apparent disparity between the doses of atropine required to inhibit methacholine- and cold air-induced bronchoconstriction may be a function of the route of administration of atropine and thus does not imply a nonmuscarinic action of atropine. These findings support the view that cold air causes bronchoconstriction via muscarinic pathways.

Sheppard, D., Epstein, J., Holtzmen, M. J., *Nadel, J. A.*, and Boushey, H. A.

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 54(1):130-133, 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco, and the Medical Service, San Francisco General Hospital, San Francisco.

CHARACTERIZATION OF *BETA* ADRENOCEPTOR SUBTYPES IN CANINE AIRWAY SMOOTH MUSCLE BY RADIOLIGAND BINDING AND PHYSIOLOGICAL RESPONSES

These researchers have investigated tracheal smooth muscle of the dog and have used [^{3}H] DHA to study the characteristics of *beta* receptors in homogenates of this tissue. For comparison, they also studied *in vitro beta* adrenergic responses in the same tissue using both exogenous *beta* agonists and electrical stimulation of sympathetic nerves. Specifically, *beta* adrenoceptor subtypes in canine tracheal smooth muscle have been investigated by radioligand binding and by physiological responses to *beta* agonists and sympathetic nerve stimulation *in vitro*. Specific binding of [^{3}H] dihydroalprenolol to tracheal smooth muscle membranes was of high affinity (K_d = 1.0 ± 0.08 nM), as in peripheral lung membranes from the same animals, but the concentration of binding sites (95.6 ± 4.7 fmol/mg of protein) was much lower in lung (532 ± 48 fmol of protein). Binding was stereoselective and agonists competed with the rank order of potency isoproterenol>epinephrine>norepinephrine, signifying a preponderance of *beta*-2 receptors. Using selective *beta* antagonists, the researchers determined the ratio of *beta*-1/*beta*-2 receptors in tracheal smooth muscle membranes to be 1:4. These and other related results suggest that most *beta* receptors in dog tracheal smooth muscle are of the *beta*-2 subtype and mediate responses to circulating catecholamines, but there is a small concentration of *beta*-1 receptors that mediate the response to neurally released norepinephrine.

Barnes, P. J., *Nadel, J. A.*, Skoogh, B-E., and Roberts, J. M.

The Journal of Pharmacology and Experimental Therapeutics 225(2):456-461, 1983.

Other support: National Institutes of Health.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

SELECTIVE GENERATION OF LEUKOTRIENE B_4 BY TRACHEAL EPITHELIAL CELLS FROM DOGS

Infiltration by neutrophils is a predominant histologic feature of acute inflammatory responses in pulmonary airways. The recent demonstration that neutrophil infiltration was localized predominantly to the epithelial layer of the airway wall in dogs breathing ozone suggested that critical inflammatory mediators were released from the epithelial cells. In the work reported here, the incubation of suspensions of canine tracheal epithelial cells of greater than 95% purity with arachidonic acid (25-200 μg/ml) for 60-120 min resulted in the generation of a maximum of 36.2 ± 9.1 picomoles of leukotriene B_4/10^6 cells, less than 2.0 picomoles of leukotrienes C_4, D_4, and E_4/10^6 cells, and 1030 ± 463, 767 ± 500, and 324 ± 100 picomoles/10^6 cells of 15-, 12-, and 5-hydroxy-eicosatetraenoic acids, respectively (mean ± SEM, n = 8). The identity of leukotriene B_4 was established by chromatographic and spectral properties, by reactivi-

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ty with mono-specific anti-plasma and by the chemotactic activity for neutrophils. Thus, the epithelium may be an important source of mediators of inflammation and hypersensitivity of pulmonary airways.

Holtzman, M. J., Aizawa, H., *Nadel, J. A.*, and Goetzl, E. J.

Biochemical and Biophysical Research Communications 114(3):1071-1076, 1983.

Other support: National Institutes of Health and the California Air Resources Board.

From the Cardiovascular Research Institute, Howard Hughes Medical Institute, and the Departments of Medicine and Physiology, University of California, San Francisco.

IMPORTANCE OF AIRWAY INFLAMMATION FOR HYPERRESPONSIVENESS INDUCED BY OZONE

As summarized in this paper, the authors studied whether ozone-induced airway hyperresponsiveness correlates with the development of airway inflammation in dogs. To assess airway responsiveness, the researchers determined increases in pulmonary resistance produced by delivering acetylcholine aerosol to the airways. To assess airway inflammation, they biopsied the airway mucosa and counted the number of neutrophils present in the epithelium. Airway responsiveness and inflammation were assessed in anesthesized dogs before ozone exposure and 1 h and 1 wk after ozone (2.1 ppm, 2 h). Airway responsiveness increased markedly at 1 h after ozone and returned to control levels 1 wk later in each of 6 dogs, but responsiveness did not change after ozone in another 4 dogs. Furthermore, dogs that became hyperresponsive also developed a marked and reversible increase in the number of neutrophils in the epithelium, whereas dogs that did not become hyperresponsive had no change in the number of neutrophils. For the group of dogs, the level of airway responsiveness before and after ozone exposure correlated closely with the number of epithelial neutrophils. The results suggest that ozone-induced airway hyperresponsiveness may depend on the development of an acute inflammatory response in the airways.

Holtzman, M. J., Fabbri, L. M., O'Byrne, P. M., Gold, B. D., Aizawa, H., Walters, E. H., Alpert, S. E., and *Nadel, J. A.*

American Review of Respiratory Disease 127:686-690, 1983.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and the California Air Resources Board.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

AUTORADIOGRAPHIC LOCALIZATION OF AUTONOMIC RECEPTORS IN AIRWAY SMOOTH MUSCLE

Using experimental conditions that proved to be optimal for specific receptor binding, these investigators have studied the distribution of alpha-adrenergic, beta-adrenergic and muscarinic receptors in smooth muscle of airways from trachea to terminal bronchioles. Autoradiographic methods were used to determine the distribu-

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

tion of autonomic receptors in airway smooth muscle of ferret from trachea to terminal bronchioles; [^{3}H] dihydroalprenolol, [^{3}H] prazosin, and [^{3}H] quinuclidinyl benzilate were used to label beta-adrenergic, alpha-adrenergic, and muscarinic receptors, respectively, using experimental conditions that gave maximal specific receptor binding. Marked differences were found in the longitudinal distribution of each receptor and in distribution of the various receptors in each caliber airway. Beta-receptors were present in high density throughout the airways, with the highest density in bronchioles. Alpha-receptors were sparse in large airways but numerous in small bronchioles, whereas cholinergic receptors were numerous in bronchial smooth muscle, sparse in proximal bronchioles and almost absent from distal bronchioles. This method may be useful in studying alterations of autonomic receptor distribution in small and large airways after experimental manipulation and in disease.

Barnes, P. J., Basbaum, C. B. and *Nadel, J. A.*

American Review of Respiratory Disease 127:758-762, 1983.

Other support: National Institutes of Health.

From the Cardiovascular Research Institute and the Departments of Anatomy and Medicine, University of California, San Francisco.

ANTIHISTAMINIC VERSUS ANTICHOLINERGIC EFFECTS OF ATROPINE ON CANINE TRACHEALIS MUSCLE

The purpose of this study was to reexamine the antihistaminic and anticholinergic effects of atropine in experiments designed to eliminate possible problems. To determine antihistaminic versus anticholinergic effects of atropine in airway smooth muscle, the investigators used an *in vitro* preparation of canine trachealis muscle strips and determined atropine's effect on contractile responses induced by histamine or by electrical field stimulation of cholinergic nerves. In the first series of experiments, 53 strips had initial responses to field stimulation determined and were then randomly assigned to a control group or to a group treated with atropine before field stimulation was repeated and histamine was given. Atropine in concentrations of 10^{-8}, 10^{-7}, and 10^{-6} M decreased the response to field stimulation to 61.4, 10.5 and 0% of the initial response, respectively, but had no effect on the responses to histamine. In the second series of experiments, 24 strips were treated with indomethacin to prevent histamine tachyphylaxis; these strips had initial responses to both field stimulation and histamine determined and were then assigned to a control group or to a group treated with atropine before field stimulation and histamine were repeated. In these experiments, a concentration of atropine (10^{-6} M), which again completely blocked the response to field stimulation, still had no effect on histamine-induced contraction. The researchers conclude that atropine in a concentration that completely blocks the response to cholinergic nerve stimulation has no antihistaminic effect.

Skoogh, B.-E., *Nadel, J. A.*, Fabbri, L. M., Sheppard, D., and Holtzman, M. J.

American Review of Respiratory Disease 128:603-608, 1983.

Other support: National Heart, Lung and Blood Institute and the Fisons Corporation.

From the Cardiovascular Research Institute and the Departments of Medicine and Physiology, University of California, San Francisco.

TIME COURSE OF AIRW OZONE IN DOGS

In the present study, hyperresponsiveness in an siveness before ozone expo To assess responsiveness, response curves of increas delivered to the airways vs with the dogs awake and at the nose and mouth at a le ppm. For both acetylcholi airway responsiveness incr degree 1 day later and retur authors' previous studies ir ness occurs shortly after ex effect is linked to an acute

Holtzman, M. J., Fabbri, Aizawa, H., and *Nadel, J.*

Journal of Applied Physiol 1983.

Other support: National H

From the Cardiovascular Physiology, University Ca

NEURAL CONTROL OF

Major advances have airway secretion, due in lar cell biology, physiology, airway submucosal glands and are regulated by vagal nonadrenergic noncholine increase gland secretion b cells. Stimulated secretion tic properties. Beta-adren little fluid, high concentra and higher viscosity and lo adrenergic stimulation cau tions, high lysozyme conc pletes granules from serou mucin secretion, probably

Nadel, J. A.

European Journal of Resp

Other support: U. S. Pub Fisons Corporation.

From the Cardiovascular R ogy, University of Califor

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TIME COURSE OF AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE IN DOGS

In the present study, the authors examined the time course of ozone-induced hyperresponsiveness in anesthetized dogs. To do this, they assessed airway responsiveness before ozone exposure and then at 1 h, 1 day, and 1 wk after ozone exposure. To assess responsiveness, the researchers anesthetized the dogs and obtained dose-response curves of increasing concentrations of acetylcholine or histamine aerosols delivered to the airways vs. pulmonary resistance. Ozone exposures were carried out with the dogs awake and at rest in an exposure chamber for 2 h breathing either through the nose and mouth at a level of 2.2 ppm or through a tracheostomy at a level of 1.0 ppm. For both acetylcholine and histamine and for both routes of ozone delivery, airway responsiveness increased most markedly at 1 h after ozone, increased to a lesser degree 1 day later and returned to control levels by 1 wk. The results are similar to the authors' previous studies in humans that showed that ozone-induced hyperresponsiveness occurs shortly after exposure and is rapidly reversible and suggest that the ozone effect is linked to an acute inflammatory response in the airways.

Holtzman, M. J., Fabbri, L. M., Skoogh, B.-E., O'Bryne, P. M., Walters, E. H., Aizawa, H., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 55(4):1232-1236, 1983.

Other support: National Heart, Lung and Blood Institute and the Fisons Corporation.

From the Cardiovascular Research Institute and the Departments of Medicine and Physiology, University California, San Francisco.

NEURAL CONTROL OF AIRWAY SUBMUCOSAL GLAND SECRETION

Major advances have occurred recently in the understanding of the processes of airway secretion, due in large part to the application of modern techniques of anatomy, cell biology, physiology, biochemistry, and pharmacology. This report notes that airway submucosal glands occupy a substantial volume of the large conducting airways and are regulated by vagal muscarinic nerves, alpha- and beta-adrenergic nerves, and nonadrenergic noncholinergic nerves. Vagal nerves modulate various reflexes that increase gland secretion by stimulating release of granules from mucous and serous cells. Stimulated secretions are unaltered from baseline in biochemical and viscoelastic properties. Beta-adrenergic stimulation releases secretions containing relatively little fluid, high concentrations of protein and sulfur, low concentrations of lysozyme and higher viscosity and lower elasticity, and selectively depletes mucous cells. Alpha-adrenergic stimulation causes high fluid flows with low protein and sulfur concentrations, high lysozyme concentrations and low apparent viscosity, and selectively depletes granules from serous cells. Nonadrenergic noncholinergic nerves also stimulate mucin secretion, probably by releasing vasoactive intestinal peptide.

Nadel, J. A.

European Journal of Respiratory Disease 64(suppl 128):322-326, 1983.

Other support: U. S. Public Health Service, Vick Division Research, Inc., and the Fisons Corporation.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

NEUROPEPTIDE TYROSINE (NPY): A NEWLY DISCOVERED PEPTIDE IS PRESENT IN THE MAMMALIAN RESPIRATORY TRACT

Neuropeptide tyrosine (NPY), a newly discovered peptide known to modulate blood vessel diameter and smooth muscle tone, has been found in many mammalian organs. Its distribution is similar to that of sympathetic nerve fibers and NPY immunoreactivity has been found in noradrenergic ganglion cells. In a study of the respiratory tract of four mammalian species — man, cat, guinea pig, and rat — NPY immunoreactivity has been localized to nerve fibers. NPY immunoreactive nerve fibers were found in the adventitia of blood vessels and in the airway smooth muscle. Its distribution was strikingly similar to that of sympathetic nerve fibers as demonstrated by dopamine-β-hydroxylase antibodies. The mean (SD) concentrations of NPY in the guinea pig respiratory tract, as determined by radioimmunoassay of tissue extracts, were: upper trachea 3.3 (0.7), lower trachea 2.0 (0.5), and major bronchus 3.5 (1.1) pmol/g. During developmental studies in man, NPY immunoreactive nerve fibers were first observed at 20 weeks' gestation in the trachea, and fibers gradually extended down into the intrapulmonary airways after birth. NPY immunoreactive nerve fibers have a distribution and developmental pattern similar to that of sympathetic nerve fibers in the respiratory tract. The finding of NPY immunoreactivity in nerve fibers in the mammalian respiratory tract adds to the growing number of peptides having potent biological actions found in this organ, and shows that the lung possesses a rich peptidergic system that may influence pulmonary function.

Sheppard, M. N., *Polak, J. M.*, Allen, J. M. and Bloom, S. R.

Thorax 39:326-330. 1984.

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.

TWO PROTEINASE INHIBITORS ASSOCIATED WITH PERITONEAL MACROPHAGES

In this study, the investigators document and characterize two proteinase inhibitors associated with guinea pig perinoneal macrophages. Results show that inhibition is dose-dependent, lost on heating and detected in the presence of excess albumin. On incubation of macrophage culture medium with ^{125}I-elastase, two complexes of $M_r = 78,000$ and 66,000 are generated which are stable to heating in sodium dodecyl sulfate, indicating covalent association. ^{125}I-Trypsin forms two complexes of similar molecular weight, and cross-inhibition experiments demonstrated that elastase and trypsin interact with the same two macrophage inhibitors. For comparison, elastase inhibitors in guinea pig plasma and cell-free peritoneal fluid were also examined. In summary, the inhibitor which forms the $M_r = 66,000$ complex with ^{125}I-elastase has been tentatively named MPI (macrophage proteinase inhibitor). MPI can be obtained free of α_1PI by culturing macrophages for 1 h. α_1PI is released into the medium during the 1st h of culture and thereafter is no longer detectable on intact macrophages, in subsequent culture media or in cell lysates. These findings suggest that plasma α_1PI is present in peritoneal fluid in high concentrations and is absorbed onto or into macrophages. MPI, on the other hand, appears to be a macrophage product, since it is secreted by macrophages after 1 or 17 h in culture and is present in lysates of macrophages precultured for 1 or 17 h.

Remold-O'Donnell, E

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Remold-O'Donnell, E. and Lewandroski, K.

The Journal of Biological Chemistry 258(5):3251-3257, 1983.

Other support: National Cancer Institute.

From the Center for Blood Research and the Department of Biological Chemistry, Harvard Medical School, Boston.

NEUTROPHILS AND THE ADULT RESPIRATORY DISTRESS SYNDROME

This paper reviews the potential role of neutrophils and their products in the pathogenesis of the acute lung injury characteristic of adult respiratory distress syndrome (ARDS). Neutrophils can elaborate O_2-derived products, proteolytic enzymes and arachidonate products. These substances have dramatic effects on experimental lung preparations. The observation that neutrophils accumulate in the lungs of ARDS patients seems clear, but this finding is obviously not specific for or diagnostic of ARDS. Therefore, despite the presence of potentially toxic neutrophils in the lungs of these patients, the specific role, if any, of neutrophils in clinical ARDS remains to be demonstrated. While basic observations must continue to be made in experimental models, an equal effort is needed to determine if experimental mechanisms of lung injury have any relationship to the pathophysiology of critically-ill ARDS patients. Only when this link is convincingly made can one begin to translate experimental findings into clinical methods for diagnosis and therapy. It is conceivable that some manipulation of neutrophil behavior could favorably affect the pathophysiology of ARDS. But because inflammation is so intimately involved in the normal processes of host defense and tissue injury and repair, therapeutic interventions that affect the inflammatory cascade could be double-edged swords and should only be studied in the most careful fashion. Given the frequency and lethality of ARDS, the cost of conducting such studies seems justified.

Tate, R. M. and *Repine, J. E.*

American Review of Respiratory Disease 128:552-559, 1983.

Other support: National Institutes of Health, American Heart Association of Wyoming, and the Kroc, Hill, Swan, and Kleberg Foundations.

From the Division of Pulmonary Sciences and the Department of Internal Medicine, University of Colorado Health Sciences Center, Denver.

MACROPHAGE EFFECTOR FUNCTION IN PULMONARY OXYGEN TOXICITY: HYPEROXIA DAMAGES AND STIMULATES ALVEOLAR MACROPHAGES TO MAKE AND RELEASE CHEMOTAXINS FOR POLYMORPHONUCLEAR LEUKOCYTES

Macrophages synthesize many secretory products *in vitro* but the stimuli for their production and their pathophysiologic significance *in vivo* are largely unknown. In the present investigation, the authors found that hyperoxia damaged rabbit alveolar macrophages (AM) *in vitro* as manifested by decreased cell numbers, increased lactate dehydrogenase release, and the development of ultrastructural abnormalities that resembled those seen in AM *in situ* or lavaged from lung of rabbits exposed to hyperoxia

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

in vivo. Hyperoxia also stimulated cultured rabbit AM to release chemotaxins for polymorphonuclear leukocytes (PMN) that were similar in molecular weight to chemotaxins obtained from lung lavages of rabbit exposed to hyperoxia *in vivo*. These results suggest that alveolar macrophage secretory products may play a physiologically relevant role in recruitment of PMN to the lungs in pulmonary oxygen toxicity.

Harada, R. N., Vatter, A. E. and *Repine, J. E.*

Journal of Leukocyte Biology 35:373-383, 1984.

Other support: American Heart Association, American Lung Association, National Institutes of Health, and the Kroc, Hill, Swan and Kleberg Foundations.

From the Webb-Waring Lung Institute and the Pulmonary Division of the University of Colorado Health Sciences Center, Denver.

INTACT HUMAN ERYTHROCYTES PREVENT HYDROGEN PEROXIDE-MEDIATED DAMAGE TO ISOLATED PERFUSED RAT LUNGS AND CULTURED BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS

Acute edematous lung injury, such as that seen in the adult respiratory distress syndrome (ARDS), is an important clinical problem whose pathophysiology is poorly defined. However, recent evidence suggests that toxic oxygen metabolites may contribute to endothelial cell injury and acute edematous lung injury. In this study, addition of untreated or glutaraldehyde-fixed human erythrocytes decreased hydrogen peroxide (H_2O_2)-mediated acute edematous injury in isolated rat lungs, H_2O_2-induced damage to cultured bovine pulmonary artery endothelial cells, and H_2O_2-dependent oxidation of reduced cytochrome C *in vitro*. RBC scavenging of H_2O_2 appeared to be dependent on intracellular glutathione and/or catalase activities. The results suggest that intact erythrocytes can scavenge H_2O_2 and, as a result, protect the lung and possibly other tissues from damage.

Toth, K. M., Clifford, D. P., Berger, E. M., White, C. W., and *Repine, J. E.*

Journal of Clinical Investigation 74:292-295, 1984.

Other support: National Institutes of Health, American Heart Association, American Lung Association, and the Swan, Hill, Kleberg, and R. J. Reynolds Foundations.

From the Departments of Medicine and Pediatrics, and the Webb-Waring Lung Institute, University of Colorado Medical Center, Denver.

OXYGEN METABOLITES STIMULATE THROMBOXANE PRODUCTION AND VASOCONSTRICTION IN ISOLATED SALINE-PERFUSED RABBIT LUNGS

Generation of reactive oxygen metabolites, thromboxane increases and vasoconstriction have been implicated in the pathogenesis of acute edematous lung injury, such as that seen in patients with the Adult Respiratory Distress Syndrome (ARDS), but their interactions are unknown. The investigators hypothesized that reactive O_2 products would stimulate arachidonic acid metabolism in lungs and that vasoactive products of arachidonate, such as the potent vasoconstrictor thromboxane A_2, might then

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mediate O_2-metabolite-induced pulmonary vasoconstriction. They found that O_2 metabolites generated by injection of purine plus xanthine oxidase caused increases in mean pulmonary artery perfusion pressure (27 ± 4mmHg) in isolated perfused lungs. In addition, purine plus xanthine oxidase also caused 30-fold increases in perfusate levels of thromboxane B_2 (the stable metabolite of thromboxane A_2) compared with only two-fold increases in 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin). Moreover, prior addition of catalase inhibited both vasoconstriction and the thromboxane B_2 production seen in isolated lungs following injection of purine plus xanthine oxidase. Similarly, pretreatment with cyclooxygenase inhibitors, either aspirin or indomethacin, also completely blocked thromboxane generation and markedly attenuated pressor responses usually seen after purine plus xanthine oxidase. Furthermore, imidazole, a thromboxane synthetase inhibitor, also decreased O_2-metabolite-induced thromboxane generation and vasoconstriction. These results suggested that thromboxane generation might participate in O_2-metabolite-induced vasoconstriction. However, since a significant correlation between thromboxane levels and the degree of vasoconstriction could not be demonstrated, and since addition of superoxide dismutase reduced thromboxane but did not affect the intensity of vasoconstriction, it is possible that thromboxane is not the only vasoactive mediator in this model. The researchers conclude that exposing lungs to O_2 metabolites results in thromboxane generation and that thromboxane is a major mediator of oxidant-induced vasoconstriction.

Tate, R. M., Morris, H. G., Schroeder, W. R. and *Repine, J. E.*

Journal of Clinical Investigation 74:608-613, 1984.

Other support: American Heart Association of Wyoming, National Institutes of Health, and the Swan, Hill, Kleberg, Roche, Thrombrands and Proctor and Gamble Foundations.

From Webb-Waring Lung Institute, and Departments of Medicine and Pediatrics, University of Colorado Health Science Center, Denver.

BRONCHIAL LAVAGE PROTEINS AS CORRELATES OF HISTOPATHOLOGIC AIRWAY CHANGES IN HEALTHY SMOKERS AND PATIENTS WITH PULMONARY CARCINOMA

Cigarette smoking is known to be an important etiologic factor in several lung diseases; however, the number of smokers who develop these diseases represents a small segment of the smoking population. It is possible that evidence of inhalation-induced injury to bronchial epithelial cells of smokers will be reflected in the proteinaceous products of these cells, thereby identifying a high-risk subgroup. The investigations have tested this hypothesis by analysis of 2 proteins, free secretory component (FSC) and the keratins, in lavage fluids obtained from 4 groups of subjects: 30 normal nonsmokers, 15 asymptomatic smokers, 22 symptomatic smokers and 40 carcinoma patients. Among symptomatic smokers, FSC relative to total protein (FSC/TP) was depressed compared with that in nonsmokers and asymptomatic smokers. The keratins were detected only in symptomatic smokers and correlated with pack/years of smoking history ($p = 0.017$). Carcinoma patients had depressed FSC/TP and detectable keratin (33 of 38 patients studied). Lung sections from carcinoma patients studied immunohistochemically revealed an apparent inverse relationship between tissue FSC and keratins. This inverse relationship was borne out by analysis of these proteins in the

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lavage fluid of cancer patients ($r = -0.4$, $p = 0.04$). Thus, in cancer patients, immunohistochemical evidence of airway injury correlates with bronchial lavage levels of mucosal epithelial cell proteins. It is possible that smokers with altered levels of these proteins may be the ones at increased risk of smoking-associated lung disease.

Merrill, W. W., Barwick, K. W., Madri, J., Strober, W., Matthay, R. A., Olchowski, J., Naegel, G., and *Reynolds, H. Y.*

American Review of Respiratory Disease 130(5):905-909, 1984.

Other support: National Heart, Lung and Blood Institute and the American Cancer Society.

From the Pulmonary Section, Department of Medicine, Veterans Administration Medical Center, West Haven, the Pulmonary Section, Department of Medicine and Department of Pathology, Yale University School of Medicine, New Haven, CT; and the Clinical Immunology Section, National Cancer Institute, Bethesda, MD.

ELASTIN PRODUCTION BY CULTURED CALF PULMONARY ARTERY ENDOTHELIAL CELLS

The apposition of endothelium to elastic fibers in large arteries and in the gas exchange regions of the lung suggests that endothelial cells have the capacity to synthesize elastin, the integral component of the elastic fiber. In this report, the authors show that endothelial cells isolated from calf pulmonary artery synthesize tropoelastin under appropriate culture conditions. This was demonstrated by radioimmunoassay for soluble elastin, by immunoprecipitation of a tropoelastin-like product from cell culture medium, and by immunofluorescent microscopy utilizing antielastin IgG. Results indicate that endothelial cell tropoelastin shows many similarities with tropoelastin synthesized by other bovine cell types, including comigration on SDS-PAGE with tropoelastin from fibroblasts, chondroblasts, and smooth muscle cells as a single-chain protein with an apparent molecular weight of approximately 70,000. In addition, the cross-reactivity of tropoelastins from endothelial cells, smooth muscle cells and ear chondroblasts with antiserum to ligament elastin indicates close homology between primary elastin gene products in the various elastin-secreting cell types. These results provide compelling evidence that cultured bovine aorta endothelial cells produce elastin when cultured in medium conditioned by smooth muscle cells, and suggest a possible role for smooth muscle cells in the regulation of elastin production by endothelial cells.

Mecham, R. P., Madaras, J., McDonald, J. A., and *Ryan, U.*

Journal of Cellular Physiology 116:282-288, 1983.

Other support: American Heart Association, Missouri Affiliate, U. S. Public Health Service, and the American Heart Association.

From the Pulmonary Division, Washington University School of Medicine at the Jewish Hospital of St. Louis, and the Department of Medicine, University of Miami, Miami, FL.

ENDOTHELIAL CELL

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ENDOTHELIAL CELLS AND INFLAMMATION

This review of endothelial cells and inflammation focuses primarily on research findings since 1979, by which time effects of inflammation on the pulmonary vasculature were recognized as contributing to the formation of a protein- and cell-rich pulmonary edema fluid. On the basis of more recent studies, investigators are now in a position to consider that what happens to the endothelium as a tissue (or to endothelial cells themselves) depends in part on active reaction to injury. The response of endothelium to injury very likely accelerates vascular occlusion in the short term but may provide a basis for repair and resolution of vascular damage in the long term. In a section on mediators of the inflammatory process, attention is given here to: (1) complement components (anaphylatoxins; (2) endothelium and inactivation of the anaphylatoxins; (3) carboxypeptidase N of endothelial cells; (4) toxic oxygen products; (5) other mediators; and (6) molecular and subcellular responses of endothelium to injury. Building on this material, a hypothesis is presented which deals with the sequence of events set in motion when a sensitized animal receives intravenous antigen. While remarkable progress has been made in understanding of cellular, subcellular and molecular mechanisms of endothelial injury and responses to injury, it appears certain, overall, that acute inflammation will not be understood in terms of a single mediator, all mediators taken together, or platelets, neutrophils, macrophages, or endothelial cells taken alone. Rather, improved understanding will likely come from clearer definitions of how all these components and cellular elements interact with the inciting stimulus and then with each other.

Ryan, U. S. and Ryan, J. W.

Clinics in Laboratory Medicine 3(4):577-599, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami, Miami, FL.

CULTURE OF PULMONARY ENDOTHELIAL CELLS ON MICROCARRIER BEADS

This chapter reviews the techniques available for the isolation and culture of pulmonary endothelial cells and the need and means for monitoring the quality of the cells through successive passages. Culture methods can be divided into those using enzymes and those which avoid exposure of cells to enzymes at both the isolation step and during subculture. The former are by and large routine, while the latter are still at the development stage but present new vistas to be explored. The enzymatic methods include: (1) Isolation of endothelial cells from pulmonary artery by collagenase digestion; (2) Isolation of pulmonary microvascular endothelium by retrograde perfusion with collagenase; and (3) Culture and subculture of pulmonary endothelial cells using trypsin EDTA for passaging. On the other hand, the methods avoiding exposure to proteolytic enzymes include: (1) Mechanical harvest of pulmonary artery endothelium; (2) Isolation of endothelium from the small vessels of the lungs by perfusion with cold saline and microcarrier beads; and (3) Microcarrier cultures: Long term, large-scale culture of pulmonary endothelium avoiding exposure to enzymes during passaging. Previous methods for culture of pulmonary endothelial cells have yielded an abundance of useful data, particularly in helping to confirm the role of the endothelium in the pulmonary processing of vasoactive substances. Now, in addition to their obvious advantages, (large number of cells, economy of media and of personnel), microcarrier

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bead cultures may allow researchers to study properties which do not survive long periods of culture and subculture involving exposure to proteolytic enzymes, and to study subtle physiologic modulations of integral membrane components and substructures such as caveolae.

Ryan, U. S.

In: Jaffe, E. A. (ed.): *Biology of the Endothelial Cell*, The Netherlands: Martinus Nijhoff, 1984, chap. 4, pp. 34-50.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

TRYPSIN-INDUCED AGGREGATION OF BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS CULTURED ON MICROCARRIERS

The authors of this paper studied adherence between "luminal" surfaces of pulmonary artery endothelial cells by standard aggregometry techniques widely used for measuring aggregation of platelets and granulocytes. Using suspensions of bovine pulmonary artery endothelial cells cultured on microcarrier beads, in an aggregometer, they found that trypsin caused endothelial aggregation. The aggregation response occurred at trypsin concentrations as low as 0.001%. The degree of trypsin-induced aggregation indicated by the magnitude of the change in light transmission through the endothelial suspensions was related to the trypsin concentration, reaching a maximum level of trypsin concentrations of 0.01%. The investigators conclude the trypsin, even in very low concentrations, causes adherence between "luminal" surfaces of pulmonary endothelial cells probably because the enzyme destroys cell surface proteins which are necessary to prevent intercellular adherence. The method described here may be useful for studying cell-cell interactions of endothelium.

Brigham, K. L., Meyrick, B. and *Ryan, U. S.*

Tissue & Cell 16(2):167-172, 1984.

Other support: National Institutes of Health, Hugh J. Morgan Fund for Cardiology, The John W. Cooke, Jr., and Laura W. Cooke Fund for Lung Research, and the Upjohn Company.

From the Pulmonary Circulation Center, Departments of Medicine and Pathology, Vanderbilt University School of Medicine, Nashville, TN, and the Department of Medicine, University of Miami School of Medicine, Miami, FL.

IN VIVO AUTORADIOGRAPHIC DEMONSTRATION OF β-ADRENERGIC BINDING SITES IN ADULT RAT TYPE II ALVEOLAR EPITHELIAL CELLS

In this study, adult male rats were injected intravenously with the muscarinic binding probe ^{3}H-quinuclidinyl benzilate (QNB) or the β-adrenergic probe ^{3}H-dihydroalprenolol (DHA). Other rats were pre-treated with an intraperitoneal injection of a 500-fold excess of L-isoproterenol prior to the DHA. Light microscopic autoradiography of 0.5 μm sections of lung from the QNB group demonstrated very little labeling even after 6 months' exposure. In contrast, trachealis smooth muscle from

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Smith, D. M. and Sidhu, N.

Life Sciences 34(6):519-527,

From the Department of Bio

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OF β-ADRENERGIC
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these animals contained substantial labeling. Autoradiographs of lung from rats injected with DHA demonstrated labeling which was well localized over alveolar septa and concentrated over the cytoplasm of type II cells. Quantitative analysis of labeling in the DHA groups indicated a significant reduction of labeling in animals treated with L-isoproterenol prior to DHA, in both the alveolar parenchyma in general and over type II cells. The results of this study provide morphologic evidence for the uptake and specific binding of β-adrenergic antagonists by the adult lung *in vivo*, while failing to demonstrate similar binding of a muscarinic probe. In addition, the results demonstrate specific β-adrenergic receptors on type II *in vivo* and substantiate the view of a direct effect of β-adrenergic agonists on alveolar type II cells.

Smith, D. M. and Sidhu, N. K.

Life Sciences 34(6):519-527, 1984.

From the Department of Biological Sciences, Wellesley College, Wellesley, MA.

HOW PHAGOCYTIC LEUKOCYTES MOVE

As this paper notes, a regulated, coordinated movement of the cytoplasm is essential for the function of phagocytes. In these cells, as in muscle cells, the power unit for movement consists of the contractile proteins, actin and myosin, which are concentrated in the region of the cell cortex. In the peripheral cytoplasm, actin fibers may be in a fluid state or they may form a gel network by association with a homodimeric, actin-binding protein. The reversible transformation of the cytoplasm from gel to sol is mediated by a regulatory protein called gelsolin which, when activated by micromolar concentrations of Ca^{2+}, causes shortening of actin fibers, leading to disintegration of the gel network. This gel network reforms if the Ca^{2+} concentration falls below the threshold value for the activation of gelsolin. Ca^{2+}, acting via gelsolin, is a second component in this system; it controls the order of events that start on the plasma membrane of the phagocyte in response to a stimulus, and that are then maintained by an appropriate reaction of the contractile unit. It is to be expected that the elucidation of the molecular mechanisms that release and regulate the movement of cytoplasm in the cell will permit an understanding of factors that interfere with leukocyte function.

Hartwig, J. H., Yin, H. L., and *Stossel, T. P.*

Journal of Clinical Chemistry and Clinical Biochemistry 21(9):535-544, 1983.

Other support: U. S. Public Health Service and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Harvard Medical School; Department of Medicine, Massachusetts General Hospital, Boston.

ISOLATION AND SOME STRUCTURAL AND FUNCTIONAL PROPERTIES OF MACROPHAGE TROPOMYOSIN

Tropomyosin purified from rabbit lung macrophages is very similar in structure to other nonmuscle cell tropomyosins. Reduced and denatured, the protein has two polypeptides which migrate during electrophoresis in sodium dodecyl sulfate on polyacrylamide gels with slightly different mobilities corresponding to apparent M_r's of about 30,000. Following cross-linking by air oxidation in the presence of $CuCl_2$, electrophoresis under nonreducing conditions reveals a single polypeptide of M_r

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

60,000. Macrophage tropomyosin has an isoelectric point of 4.6 and an amino acid composition similar to other tropomyosins. It contains one cysteine residue per chain. In the electron microscope, macrophage tropomyosin molecules rotary shadowed with platinum and carbon are slender, straight rods, 33 nm in length. Macrophage tropomyosin paracrystals grown in high magnesium concentrations have an axial periodicity of 34 nm. On the basis of yields from purification and from two-dimensional electrophoretic analyses of macrophage extracts, tropomyosin comprises less than 0.2% of the total macrophage protein, a molar ratio of approximately 1 tropomyosin molecule to 75 actin monomers in the cell. Macrophage tropomyosin binds to actin filaments. Macrophage, skeletal muscle and other nonmuscle cell tropomyosins inhibit the fragmentation of actin filaments by the Ca^{++}-gelsolin complex. The finding implies that tropomyosin may have a role in stabilizing actin filaments in vivo.

Fattoum, A., Hartwig, J. H. and *Stossel, T. P.*

Biochemistry 22(5):1187-1193, 1983.

Other support: U. S. Public Health Service.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

THREE-DIMENSIONAL STRUCTURE OF ACTIN FILAMENTS AND OF AN ACTIN GEL MADE WITH ACTIN-BINDING PROTEIN

This paper describes four properties for purified actin filaments and for actin assembled in the presence of macrophage actin-binding protein. To do this, purified muscle actin and mixtures of actin and actin-binding protein were examined in the transmission electron microscope after fixation, critical point drying and rotary shadowing. The three-dimensional structure of the protein assemblies was analyzed by a computer-assisted graphic analysis applicable to generalized filament networks. This analysis yielded information concerning the frequency of filament intersections, the filament length between these intersections, the angle at which filaments branch at these intersections, and the concentration of filaments within a defined volume. Purified actin at a concentration of 1 mg/ml assembled into a uniform mass of long filaments which overlap at random angles between 0 and 90. Actin in the presence of macrophage actin-binding protein assembled into short, straight filaments, organized in a perpendicular branching network. The distance between branch points was inversely related to the molar ratio of actin-binding protein to actin. This distance was what would be predicted if actin filaments grew at right angles off nucleation sites on the two ends of actin-binding protein dimers and then annealed. The results suggest that actin in combination with actin-binding protein self-assembles to form a three-dimensional network resembling the peripheral cytoskeleton of motile cells.

Niederman, R., Amrein, P. C., and Hartwig, J. (*Stossel, T. P.*)

The Journal of Cell Biology 96:1400-1413, 1983.

Other support: U. S. Public Health Service, the Edwin S. Webster Foundation and the Veterans Administration.

From the Hematology-Oncology Unit, Department of Medicine, Massachusetts General Hospital, Boston, and the Veterans Administration Medical Center, West Roxbury, MA.

ISOLATION OF ACTIN-BINI
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Corwin, H. L. and Hartwig,

Developmental Biology 99:61

Other support: U. S. Public
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Zaner, S. K. and *Stossel, T*

The Journal of Biological

Other support: U. S. Publ

From the Hematology-Onc
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ISOLATION OF ACTIN-BINDING PROTEIN AND VILLIN FROM TOAD OOCYTES

Two actin-modulating proteins have been purified from toad oocytes. A high-molecular weight protein, similar in structure and function to macrophage actin-binding protein, accounts for the isotropic actin-crosslinking activity in oocyte homogenates. A calcium-dependent activity in toad oocyte homogenates which shortens actin filaments is accounted for by a 95,000-dalton protein which resembles villin, an actin-severing and -bundling protein of avian epithelial brush borders. In the presence of high ($\geq \mu M$) calcium, this protein shortens actin filaments in a concentration-dependent fashion and stimulates filament assembly when added to monomeric actin. In the absence of calcium the protein promotes the formation of actin filament bundles. Therefore, in the toad oocyte actin can be crosslinked into a network of actin-binding protein. Calcium regulation of the actin network may be mediated by villin. These results are different from those reported in echinoderm eggs.

Corwin, H. L. and Hartwig, J. H. (*Stossel, T. P.*)

Developmental Biology 99:61-74, 1983.

Other support: U. S. Public Health Service and the Elsie O. and Philip D. Sang Foundation.

From the Hematology-Oncology and Renal Units, Harvard Medical School, Department of Medicine, Massachusetts General Hospital, Boston.

PHYSICAL BASIS OF THE RHEOLOGIC PROPERTIES OF F-ACTIN

In the study reported here, the viscoelastic properties of purified rabbit skeletal muscle actin filaments (F-actin) were measured at physiologic ionic strength and pH over a range of concentrations and filament lengths. Although F-actin demonstrated transitory elastic behavior, viscous flow was observed at longer times consistent with a high degree of filament overlap. The compliance was independent of stress over a 4-fold range, implying that the measurement did not disrupt any interfilament "bonds." The dynamic storage modulus increased monotonically with frequency over the range measured, whereas the dynamic loss modulus had a relative minimum and was always less than the dynamic storage modulus. These observations are typical of topologically constrained behavior. The absolute value of the complex dynamic viscosity of F-actin, varied as the -0.8 power of the frequency and at a frequency of 0.1 radians/s was proportional to the product of the weight average filament length raised to the 0.7 power and the concentration. The experimental data agreed well with the predictions of a theory of the rheologic behavior of stiff rods in semidilute solutions. We conclude that the mechanical behavior of pure F-actin solutions can be explained on the basis of the mutual topologic constraints to diffusion of long stiff rods which do not otherwise interact.

Zaner, S. K. and *Stossel, T. P.*

The Journal of Biological Chemistry 258(18):11004-11009, 1983.

Other support: U. S. Public Health Service.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

NEW MECHANISM-BASED SERINE PROTEASE INHIBITORS: INHIBITION OF HUMAN LEUKOCYTE ELASTASE, PORCINE PANCREATIC ELASTASE, HUMAN LEUKOCYTE CATHEPSIN G, AND CHYMOTRYPSIN BY 3-CHLOROISOCOUMARIN AND 3,3-DICHLOROPHTHALIDE

Mechanism-based irreversible inhibitors, which have been reported for porcine pancreatic (PP) elastase and bovine pancreatic chymotrypsin A, include halo enol lactones and 6-chloropyrones. Human leukocyte (HL) elastase and cathepsin G are related serine proteases which are involved in the connective tissue destruction that occurs in emphysema and various inflammatory diseases. Both enzymes are inhibited reversibly by heterocyclic structures such as benzoxazinones and benzisothiazolinones, and this suggested that heterocycles containing masked reactive functionalities might act as mechanism-based irreversible inhibitors for HL elastase and cathepsin G. Therefore, these authors prepared 3-chloroisocoumarin (3-chloro-1H-2-benzopyran-1-one) (1) and 3,3-dichlorophthalide (2) and found them to be potent inhibitors of several serine proteases. In summary, evidence presented here indicates that 1 and 2 are mechanism-based irreversible inhibitors of serine proteases. These are the first demonstrated examples of enzyme-activated inhibitors of HL elastase and cathepsin G. These enzymes have been noted to be major contributors to elastin destruction observed in emphysema. These inhibitors and similar structures may have considerable pharmacologic potential as inhibitors *in vivo*.

Harper, J. W., Henni, K. and *Powers, J. C. (Travis, J.)*

Journal of the American Chemical Society 105:6518-6520, 1983.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

MAMMALIAN TISSUE TRYPSIN-LIKE ENZYMES: COMPARATIVE REACTIVITIES OF HUMAN LUNG TRYPTASE, AND BOVINE TRYPSIN WITH PEPTIDE 4-NITROANILIDE AND THIOESTER SUBSTRATES

The subsite specificity of human lung and skin tryptase (trypsin-like enzyme) has been studied at pH 7.5 using 17 amino acid and dipeptide thioester substrates and 14 tripeptide 4-nitroanilide substrates. The reactivity and specificity of the human tryptases were compared with bovine trypsin and other trypsin-like enzymes. Neither tryptase was similar to either kallikrein or factor XII, (Hageman factor). The skin enzyme was the most reactive as measured by the specificity constant k_{cat}/K_M. The best substrate was benzyloxycarbonyl(Z)-Lys-Arg-S-$CH_2CH(CH_3)_2$ which had a k_{cat}/K_M value of 59,000,000 $M^{-1}S^{-1}$. Only a single substrate, Z-Glu-Phe-Arg-4-nitroanilide, was slightly more reactive with the lung tryptase. Both enzymes have extended substrate-binding sites and proline residues at P_3 substantially decrease k_{cat}/K_M. Both enzymes preferred the tripeptide 4-nitroanilides with a P_2 Gly residue over Phe, and both favored the substrate Z-Lys-Gly-Arg-4-nitroanilide over similar substrates containing six other representative amino acid residues at P_2. The lung enzyme was inhibited over three times faster by p-amidinophenyl-methanesulfonyl fluoride than the skin enzyme. The preference of the skin tryptase for substrates with two terminal basic residues indicates that this enzyme could process prohormones and proproteins which contain this structural feature at the cleavage site. The substrates reported in

this paper should be use
function of tryptases.

Tanaka, T., McRae, B. J.
Powers, J. D. (Travis, J.)

The Journal of Biological

Other support: National In

From the School of Chemis
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Clinical Chemistry 29(3):

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this paper should be useful for the further characterization of the physiologic function of tryptases.

Tanaka, T., McRae, B. J., Cho, K., Cook, R., Fraki, J. E., *Johnson, D. A.*, and *Powers, J. D.* (*Travis, J.*)

The Journal of Biological Chemistry 258(22):13552-13557, 1983.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta; the Department of Dermatology, University of Kuopio, Kuopio, Finland; and the Department of Biochemistry, College of Medicine, East Tennessee State University, Johnson City.

III. *Heart and Circulation*

INTERLABORATORY PROFICIENCY SURVEY OF HIGH-DENSITY LIPOPROTEIN CHOLESTEROL MEASUREMENT

Accuracy in the quantification of high-density lipoprotein (HDL) cholesterol is important because measured values are generally interpreted, not in relation to a laboratory's own reference interval but in relation to epidemiological data, when a patient's risk of coronary artery disease is being assessed. Likewise, good precision is important because relatively large differences in risk are predicted by small changes in HDL cholesterol. According to the work presented here, proficiency surveys of Seattle-area laboratories suggest only slight improvement in overall performance in HDL measurement between 1978 and 1982, although the reported workload for HDL has increased by 15%. The mean interlaboratory SD was 64 mg/L in 1982, compared with 79 mg/L in 1978-79. Of the individual laboratory results in the current survey, 39% deviated by more than 50 mg/L from target values as compared with 37% in 1978-79. The discrepant values were primarily ascribable to method inaccuracy. For within-run precision, 80% of laboratories in 1982 had SDs of <30 mg/L, *vs.* 70% in 1978. The 1982 survey included a lyophilized serum prepared by spray freezing in bulk lyophilization (Hyland Omega), identical to the pools used in the College of American Pathologists Comprehensive Chemistry Survey, and five pools of frozen plasma. Interlaboratory variation and biases for the Omega pool were similar to those for the frozen pools.

Warnick, G. R., Benderson, J. M. and *Albers, J. J.*

Clinical Chemistry 29(3):516-519, 1983.

From the Northwest Lipid Research Clinic, University of Washington School of Medicine, Seattle.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

HUMAN PLATELET-DERIVED GROWTH FACTOR (PDGF): AMINO-TERMINAL AMINO ACID SEQUENCE

Elucidation of the primary structure of platelet-derived growth factor (PDGF) will help provide an understanding of the molecular basis for its function and its relation to other polypeptide growth factors, serum proteins, and platelet factors. This paper describes the results of amino-terminal amino acid sequence analysis of both active PDGF and the major, inactive peptides derived from it by disulfide reduction. To do this, PDGF obtained from outdated human platelets was subjected to amino-terminal sequence analysis by automated Edman degradation. Despite the apparent presence of limited proteolytic degradation of the protein derived from this method, the sequence analysis revealed two primary peptide sequences and suggests that active PDGF is composed of two, possibly homologous peptides linked by a disulfide bond or bonds.

Antoniades, H. N. and Hunkapiller, M. W.

Science 220:963-965, 1983.

Other support: Weingart Foundation and the National Cancer Institute.

From the Center for Blood Research and Department of Nutrition, Harvard University School of Public Health, Boston, and the Division of Biology, California Institute of Technology, Pasadena.

SIMIAN SARCOMA VIRUS *onc* GENE, v-*sis*, IS DERIVED FROM THE GENE (OR GENES) ENCODING A PLATELET-DERIVED GROWTH FACTOR

Recent investigations of the genetic alterations that cause normal cells to become malignant have focused on a small set of cellular genes. Acute transforming retroviruses have substituted viral genes necessary for replication with these discrete segments of host genetic information. When incorporated within the retroviral genome, these transduced cellular sequences, termed *onc* genes, acquire the ability to induce neoplastic transformation. The profound cellular alterations induced by the activated cellular transforming genes have some similarities to the growth promoting actions of hormones and growth factors. In this paper, it is demonstrated that the transforming protein of a primate sarcoma virus and a platelet-derived growth factor are derived from the same or closely related cellular genes. This conclusion is based on the demonstration of extensive sequence similarity between the transforming protein derived from the simian sarcoma virus *onc* gene, v-*sis*, and a human platelet-derived growth factor. The mechanism by which v-*sis* transforms cells could involve the constitutive expression of a protein with functions similar or identical to those of a factor active transiently during normal cell growth.

Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devard, S. G., Robbins, K. C., Aaronson, S. A., and *Antoniades, H. N.*

Science 221:275-277, 1983.

Other support: National Institutes of Health and the Weingart Foundation.

From the Department of Chemistry, University of California, San Diego, La Jolla; Division of Biology, California Institute of Technology, Pasadena; Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD; and the Center for Blood Research and Department of Nutrition, Harvard University School of Public Health, Boston.

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Antoniades, H. N. and Wi

Federation Proceedings 4

Other support: National I
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HUMAN PLATELET-DERIVED GROWTH FACTOR: STRUCTURE AND FUNCTION

This paper briefly reviews the physicochemical properties of human platelet-derived growth factor (PDGF) and its diverse physiological functions, which include stimulation of cell growth, cell migration, cell metabolism, and modulation of cellular membrane receptors. In summary, PDGF is a heat-stable, cationic polypeptide transported in blood in the α granules of platelets. It is released from platelets during blood clotting. PDGF has been resolved into at least two closely related active polypeptides, PDGF-I and PDGF-II, each consisting of two inactive chains linked together by disulfide bonds. PDGF stimulates the growth of normal cells in culture, including fibroblasts, arterial smooth muscle cells, and glial cells. In addition, PDGF has been shown to stimulate cell migration and many diverse metabolic functions such as amino acid transport, protein synthesis, cholesterol ester synthesis, phospholipid turnover, and prostacyclin synthesis. It modulates receptor binding of other active components such as epidermal growth factor, luteinizing hormone, low-density lipoprotein, and somotomedin C. Specific cell membrane receptors for PDGF have been demonstrated in arterial smooth muscle cells and fibroblasts.

Antoniades, H. N. and Williams, L. T.

Federation Proceedings 42:2630-2634, 1983.

Other support: National Institutes of Health and the Milton Fund, Harvard Medical School.

From the Center for Blood Research and Department of Nutrition, Harvard University School of Public Health, Boston, and the Cardiac Unit, Massachusetts General Hospital, Boston.

THE BIOCHEMICAL BASIS OF MYOCARDIAL FAILURE

In this careful review paper, it can be seen that the findings of more than a century of investigation point to a picture not of a discrete lesion, but rather of the interaction of multiple factors that converge on the common pathway of heart failure. It would seem that the pathways leading to myocardial failure can have a variety of metabolic origins. They can start from disturbances within the myocardium — the processes of energy production in mitochondria, energy utilization by the contractile proteins, or altered calcium flux in one or more of the cellular organelles involved — or they may arise because of faulty β-adrenergic regulation affecting the adrenergic component of contraction. New technology is making it possible to synthesize the contributing factors into a clinical model that will greatly enhance investigators' understanding of these mechanisms. Specifically, the chemical reactions in metabolism need to be followed inside living cells, and indeed, this is now promised through the phenomenon of nuclear magnetic resonance (NMR). As NMR spectroscopy brings the study of cardiac metabolism and biochemistry to the bedside, understanding of fundamental mechanisms of various cardiac disorders, especially of the heart failure state, should greatly increase.

Bing, R. J.

Hospital Practice September 1983, pp. 93, throughout.

From the Huntington Medical Research Institutes, Pasadena, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

THE EFFECTS OF A NEW CARDIOTONIC DRUG (TA-064) ON NORMAL AND FAILING HEART PREPARATIONS

Since Withering discovered the action of digitalis in 1785, few drugs have been introduced with prolonged inotropic effect on the failing heart. This report, however, deals with the effect of TA-064, a new positive inotropic compound, on the metabolic and dynamic function of normal and failing perfused working rabbit heart preparations. In order to increase the oxygen capacity of the perfusate, a perfluorocarbon (FC-43) oxygenated in an infant bubble oxygenator was employed. Metabolic measurements included high energy phosphate and glycolytic intermediates. Left ventricular systolic, end-diastolic pressures, dp/dt_{max}, cardiac output, coronary flow, and myocardial oxygen consumption were determined. Administration of sodium pentobarbital (50 to 100 mg) or increased pre- and afterload resulted in depression of myocardial contractility. In the nonfailing heart preparation, single injections of TA-064 resulted in marked positive inotropic effects, increased heart rate and myocardial oxygen consumption. Propranolol in concentrations of 4 ng/ml abolished the positive inotropic effect. These and other observations presented here show that TA-064 is a powerful positive inotropic agent which in the isolated perfused heart increases both heart rate and myocardial oxygen consumption. It does not influence high energy phosphate or glycolytic intermediates in heart muscle.

Bing, R. J. et al.

Current Therapeutic Research 36(1):133-147, 1984.

Other support: The Hoover Foundation and the Lindbergh Fund.

From the Department of Experimental Cardiology, Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

MICROBUBBLE DYNAMICS VISUALIZED IN THE INTACT CAPILLARY CIRCULATION

Existing techniques for two-dimensional contrast echocardiographic imaging of myocardial tissue rely on the introduction of contrast agent solutions containing microbubbles of air, which alter the acoustic impedance and produce differential echo enhancement. The two methods used in the investigators' laboratory to generate microbubbles in solutions serving as contrast agents included the widely used hand-agitation method and the newer ultrasonic microcavitation (sonication) method. The latter has been demonstrated to generate smaller and more uniform microbubbles in an *in vitro* system. This study was designed to observe, by direct microscopic examination of a cat mesentery preparation, the behavior and fate of the microbubbles in an *in vivo* system. The *in vivo* mesentery observations confirm the critical role of microbubble size in its unhindered passage through the capillary vasculature. The smaller and more uniform sonicated microbubbles passed rapidly through the microcirculation along with the red blood cells, whereas the larger microbubbles were observed to coalesce and interrupt the flow of blood and subsequently collapse or shrink.

Feinstein, S. B., Shah, P. M., *Bing, R. J.*, Meerbaum, S., Corday, E., Chang, B-L., Santillan, G., and Fujibayashi, Y.

Journal of the American College of Cardiology 4(3):595-600, 1984.

Other support: Americ
Group of Cedars-Sinai
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From the Huntington M
Cardiology, Departmer
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., Corday, E., Chang, B-L.,

-600, 1984.

Other support: American Heart Association, The Hoover Foundation, the Medallion Group of Cedars-Sinai Medical Center, the Keck Foundation, and the Eliot Corday Research Foundation.

From the Huntington Medical Research Institutes, Pasadena, CA, and the Division of Cardiology, Department of Medicine, Cedars-Sinai Medical Center, the University of California at Los Angeles School of Medicine and the Wadsworth Veterans Administration Medical Center, Los Angeles.

EFFECT OF SEROTONIN ON ALBUMIN AND LOW DENSITY LIPOPROTEIN UPTAKE IN PERFUSED RABBIT FEMORAL ARTERIES

Transport of blood plasma constituents into the arterial wall has considerable clinical implication. Increased intraluminal pressure, turbulence and shear stress are important factors in the production of vascular injury to the endothelium. Thus, uptake of low density lipoprotein (LDL) plays an important role in the development of atherosclerosis. Serotonin and other vasoactive material may result in changes in the arterial wall leading to increased uptake of albumin or LDL. The present study was conducted to determine the effect of serotonin (10^{-5} M 5-hydroxytryptamine) and serotonin-induced arterial constriction on albumin and LDL uptake rates in perfused rabbit femoral arteries. The results show that the presence of serotonin inhibits the rate of uptake of both albumin and LDL. The effect on albumin uptake does not have a direct dose response dependence and is linearly dependent on transmural pressure. In contrast, LDL uptake rates are only slightly affected by pressure. Thus, albumin and LDL uptake processes appear to be due to separate mechanisms.

Santillan, G. G., Sato, M. and *Bing, R. J.*

Angiology 35(11):694-700, 1984.

Other support: The Hoover Foundation and The Lluella Morey Murphy Foundation.

From the Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

RED CELL VELOCITY AND AUTOREGULATION IN THE CEREBRAL CORTEX OF THE CAT

This report compares the effect of changes in blood pressure on blood flow with those in red cell velocity in the cerebral cortical microcirculation of the cat. To do this, total and regional blood flows were measured during a steady-state with radioactive microspheres; a newly devised method, based on transillumination, was employed for direct visualization of the microcirculation in the cerebral cortex. Craniotomy failed to influence either autoregulation or red cell velocity under the skull opening or of the total cerebral flow. Autoregulation of total and regional cerebral flow was present. In contrast, marked pressure-dependent variations in microcirculatory red cell velocity occurred. A fall in systemic pressure was accompanied by a decrease in red cell velocity; return of pressure resulted in increased red cell velocity and in some instances, in an overshoot. Considerable inhomogeneity of red cell velocity in different microcirculatory vessels was encountered. In some cases a fall in systemic pressure led to complete disappearance of red cells.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Chang, B. L., Santillan, G. and *Bing, R. J.*

Brain Research 308:15-24, 1984.

Other support: The Hoover Foundation and The Lluella Morey Murphy Foundation.

From the Huntington Medical Research Institutes and the Huntington Memorial Hospital, Pasadena, CA.

ANTIBODIES TO THE HEPARAN SULFATE PROTEOGLYCANS SYNTHESIZED BY ENDOTHELIAL CELL CULTURES

In this paper, some of the properties of an antiserum raised against the heparan sulfate proteoglycans are described, and the implications of these findings with respect to an understanding of the biological significance of endothelial cell proteoglycans are discussed. The cell lines used for these studies, an endothelial cell line and a subendothelial cell line, were derived from the intima of rabbit aorta. Using polyacrylamide gel electrophoresis, it was seen that endothelial cell cultures derived from rabbit aorta synthesize a wide spectrum of heparan sulfate proteoglycans. The electrophoretically slower-moving heparan sulfate proteoglycans have been isolated from the endothelial cell culture medium. Antibodies to these proteoglycans species have been raised in the goat. The goat antiserum binds selectively the heparan sulfate proteoglycans that were used for the immunization and does not cross-react with the other (faster moving) species. Only a moderate level of cross-reactivity was observed with the heparan sulfate proteoglycans synthesized by another cell line (presumably smooth muscle cells) of vascular derivation. These results support the suggestion that structural differences in the heparan sulfate proteoglycans are responsible for certain differences in function between the various cell types of the vessel wall.

Buonassisi, V. and Colman, P.

Biochimica et Biophysica Acta 760:1-12, 1983.

Other support: National Institutes of Health.

From the Department of Biology Q-058, University of California, San Diego, La Jolla.

HUMAN PLASMA KALLIKREIN RELEASES NEUTROPHIL ELASTASE DURING BLOOD COAGULATION

Granules from viable human neutrophils contain a variety of enzymes known to be released into the extracellular environment. One of the more important granules is elastase, which may be responsible for proteolysis of vital structures in the lung. Since human plasma kallikrein has been shown to stimulate neutrophil chemotaxis, aggregation and oxygen consumption, the ability of kallikrein to release neutrophil elastase was investigated. Neutrophils were isolated by dextran sedimentation, and elastase release was measured by both an enzyme-linked immunosorbent assay and an enzymatic assay using *t*-butoxy-carbonyl-Ala-Ala-Pro-Val-amino methyl coumarin as the substrate. Kallikrein, 0.1-1.0 U/ml, was incubated with neutrophils that were preincubated with cytochalasin B (5μg/ml). The release of elastase was found to be proportional to the kallikrein concentration. Kallikrein released a maximum of 34% of the total elastase content. In a series of experiments carried out to determine if kallikrein

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

was a major enzyme involved in neutrophil elastase release during blood coagulation, 2.75μg of elastase were released when 10 million neutrophils were incubated in 1ml of normal plasma in the presence of 30 mM $CaCl_2$ for 90 min. In contrast, neutrophils incubated in prekallikrein-deficient or Factor XII-deficient plasma released less than half that amount of elastase. The addition of purified prekallikrein to prekallikrein-deficient plasma restored neutrophil elastase release to normal levels. Moreover, release of elastase was enhanced in plasma deficient in C1-inhibitor, the major plasma inhibitor of kallikrein. These and other data suggest that kallikrein may be a major enzyme responsible for the release of elastase during blood coagulation.

Wachtfogel, Y. T., Kucich, U., James, H. L., Scott, C. F., Schapira, M., Zimmerman, M., Cohen, A. B., and *Colman, R. W.*

Journal of Clinical Investigation 72:1672-1677, 1983.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Thrombosis Research Center and the Hematology-Oncology and Pulmonary Sections, Department of Medicine, Temple University School of Medicine, Philadelphia; Graduate Hospital Research Center, Division de Rheumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Merck Sharp & Dohme Laboratories, Rahway, NJ.

CLEAVAGE OF HUMAN HIGH MOLECULAR WEIGHT KININOGEN MARKEDLY ENHANCES ITS COAGULANT ACTIVITY: EVIDENCE THAT THIS MOLECULE EXISTS AS A PROCOFACTOR

The present report demonstrates that intact high molecular weight (HMW)-kininogen is a procofactor that is activated by kallikrein. To be precise, HMW-kininogen, the cofactor of contact-activated blood coagulation, accelerates the activation of Factor XII, prekallikrein, and Factor XI on a negatively charged surface. Although prekallikrein and Factor XI circulate as a complex with HMW-kininogen, no physical association has been demonstrated between Factor XII and HMW-kininogen, nor has the order of adsorption to surfaces of these proteins been fully clarified. In this report, the investigators explore the requirements for adsorption of HMW-kininogen to a clot-promoting surface (kaolin) in purified systems as well as in normal plasma and plasma genetically deficient in each of the proteins of the contact system. In normal plasma, the adsorption of HMW-kininogen to kaolin increased as the quantity of kaolin was increased in the incubation mixture. However, the HMW-kininogen in Factor XII-deficient plasma did not absorb appreciably to kaolin. Furthermore, the quantity of HMW-kininogen from prekallikrein-deficient plasma that adsorbed to kaolin was decreased as compared with normal plasma. Since kallikrein, which is known to cleave HMW-kininogen, is generated when kaolin is added to plasma, the hypothesis was tested that proteolysis by kallikrein was responsible for the enhanced adsorption of HMW-kininogen to kaolin. The observations reported here indicate that HMW-kininogen is a procofactor that is activated by kallikrein, a product of a reaction which it accelerates. This cleavage, which enhances its association with a clot-promoting surface in a plasma environment, is an event that is necessary for expression of its cofactor activity.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Scott, C. F., Silver, L. D., Schalira, M., and *Colman, R. W.*

Journal of Clinical Investigation 73:954-962, 1984.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Thrombosis Research Center and the Hematology-Oncology Section of the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

PERSPECTIVES: SURFACE-MEDIATED DEFENSE REACTIONS: THE PLASMA CONTACT ACTIVATION SYSTEM

Observations recorded in this review paper show that, during the past two decades, considerable evidence has accrued that many of the body defenses against injury, specifically blood coagulation, the inflammatory response, classical complement pathway activation and fibrinolysis, are initiated by a common mechanism. Four proteins, namely Factor XII, prekallikrein, high molecular weight kininogen, and Factor XI, have been shown to be the major proteins required to initiate, amplify, and propagate surface-mediated defense reactions by activating $C\bar{1}$, Factor VII, prorenin and plasminogen, by stimulating neutrophils and by releasing bradykinin. Purification of each has allowed functional and immunochemical assays to be developed. The zymogens, XII, PK, and XI_1 are converted by limited proteolysis into the active serine proteases XII_a, kallikrein, and XI_a. High molecular weight kininogen is a nonenzymatic cofactor. The molecular events occurring during *in vitro* contact activation have begun to be described and the role of these pathways in certain diseases has been tentatively probed. Despite advances, new questions have been raised in tandem with the answers provided. Several clinical examples of abnormalities of the contact activation system indicate the need for further application of biochemical knowledge and immunochemical techniques to develop more sensitive assays for activation of the contact system. Emphasis should be shifted from examining the participation of this system in hemostasis to the role of surface-mediated reactions as a defense system in inflammatory diseases.

Colman, R.W.

Journal of Clinical Investigation 73:1249-1253, 1984.

Other support: National Institutes of Health and SCOR on Thrombosis.

From the Thrombosis Research Center and Hematology-Oncology Section of the Department of Medicine, Temple University School of Medicine, Philadelphia.

INACTIVATION OF FACTOR XII ACTIVE FRAGMENT IN NORMAL PLASMA: PREDOMINANT ROLE OF $C\bar{1}$-INHIBITOR

This study indicates that $C\bar{1}$-inhibitor is the major inhibitor of Factor XIIf in normal human plasma. This conclusion is supported by (a) the analysis of the kinetics of Factor XIIf inactivation in purified systems and in prekallikrein-deficient plasma, and (b) the quantitation by sodium dodecyl sulfate polyacrylamide gel electrophoresis

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de Agostini, A., Lijnen,

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(SDS-PAGE) of the Factor XIIf-inhibitor complexes formed in various plasma as the result of the inactivation of purified radiolabeled enzyme. Specifically, in purified systems, the bimolecular rate constants for the reactions of Factor XIIf with $C\bar{1}$-inhibitor, α_2-antiplasmin, and antithrombin III were 18.5, 0.91, and 0.32 $\times 10^6$ M^{-1} min^{-1}, respectively. Furthermore, SDS-PAGE analysis revealed that 1:1 stoichiometric complexes were formed between ^{125}I-Factor XIIf and each of these three inhibitors. In contrast, kinetic and SDS-PAGE studies indicated that Factor XIIf did not react with α_1-antitrypsin or α_2-macroglobulin. In prekallikrein-deficient plasma, the inactivation rate constant of Factor XIIf was 14.4 $\times 10^{-2}$/min^{-1}, a value that was essentially identical to the value predicted from the studies in purified systems. After inactivation in a normal plasma, 74% of the active ^{125}I-Factor XIIf was found to form a complex with $C\bar{1}$-inhibitor, whereas 26% of the enzyme formed complexes with α_2-antiplasmin and antithrombin III. Other observations presented in this study quantitatively demonstrate the dominant role of $C\bar{1}$-inhibitor in the inactivation of Factor XIIf in the plasma milieu.

de Agostini, A., Lijnen, H. R., Pixley, R. A., *Colman, R. W.*, and Schapira, M.

Journal of Clinical Investigation 73:1542-1549, 1984.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Division de Rheumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Center for Thrombosis and Vascular Research, Department of Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; and Hematology-Oncology Section, Department of Medicine, and Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

EFFECTS OF DETERGENTS ON THE ACTIVITY OF α_2-MACROGLOBULIN-PROTEASE COMPLEXES

The enzymatic activity of complexes of proteases, particularly chymotrypsin and elastase, with human α_2-macroglobulin (α_2M) in the presence and absence of detergents such as sodium dodecyl sulfate (SDS) and long-chain fatty acids is reported in this paper. It was found that although α_2M human leukocyte elastase (HLE) complexes have little or no elastolytic activity in the absence of detergents, elastolytic activity becomes significant in their presence at concentrations approximating that of naturally occurring fatty acids in plasma. As results show, when assayed in the presence of detergents such as SDS or sodium lineolate, apparent elastolytic activity of HLE increases almost three-fold, reaching a maximum at a detergent concentration of 1-2 mM. If the HLE is pretreated with an excess of α_2M such that in the absence of detergent all elastolytic activity is lost, and then assayed in the presence of increasing concentrations of either SDS or sodium linoleate, apparent elastolytic activity increases to that of free HLE determined in the absence of detergent. The stimulatory effects of detergents appear to be specific for the α_2M-HLE complex as SDS, sodium lineolate, and sodium oleate inhibited amidase activity of both free and complexed HLE. In each instance, the detergent concentration to reduce amidase activity was approximately 50 μM. These data are consistent with a model in which proteolytic activity is associated with the α_2M-protease complex rather than one in which proteo-

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

lytic activity is due to dissociated enzyme. The authors suggest this phenomenon may be important in the pathogenesis of emphysema and other inflammatory diseases.

Finlay, T. H. et al.

Annals of the New York Academy of Sciences 421:340-350, 1983.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York, and the Department of Medicine and Chest Service, Bellevue Hospital, New York.

ACTIVE INTERMEDIATES AND CARCINOGENESIS

In the study presented here, the neoplastic transformation of Balb/c 3T3 cells has been shown to be induced by diethylstilbestrol, estrone, ethylnylestradiol, and estradiol. Moxestrol and estriol, which are poor substrates for catechol estrogen biosynthesis, were not active as transforming agents of these cells. The relative rates of catechol estrogen formation, with ethynylestradiol>estradiol>11β-methylestradiol>11β-ethylestradiol>moxestrol, paralleled the observed frequency of neoplastic transformation. The fact that moxestrol and the 11β-methyl and 11β-ethyl analogs are more potent estrogens *in vivo* than estradiol supports the investigators' hypothesis that the oncogenic potential of an estrogen is related to its relative rate of catechol estrogen formation by the microsomal activating system of the target cells rather than to its activity as a hormone. Also, three of the possible α,β-unsaturated epoxyenone intermediates of estradiol metabolism have been synthesized from 19-nortestosterone and unambiguously characterized. These epoxyenones are approximately 10 to 20 times more potent than estradiol as transforming agents. It is not yet known if their transforming ability is attributable to their direct reaction with DNA to form DNA adducts or to their intracellular conversion to catechol estrogens before DNA damage presumably occurs. In addition, it was seen here that the microsomal activating system of a continuously cultured line of human mammary tumor cells can synthesize catechol estrogens. This MCF-7 cell activating system has been used in these researchers' efforts to isolate an activated intermediate of estradiol metabolism. Their preliminary results provide evidence for the formation of a metabolite which, after acetylation, has the chromatographic mobility of 17β-acetoxy-1β,2β-epoxyestra-4-en-3-one.

Purdy, R. H., Goldzieher, J. W., *Le Quesne, P. W.*, Abdel-Baky, S., Durocher, C. K., Moore, P. H., Jr., and Rhim, J. S.

In: Merriam, G. R. and Lipsett, M. B. (eds.): *Catechol Estrogens*, New York: Raven Press, 1983, pp. 123-140.

Other support: National Cancer Institute, American Cancer Society, and the Joe and Jessie Crump Fund of Fort Worth, TX.

From the Department of Organic and Biological Chemistry, Southwest Foundation for Research and Education, San Antonio, TX; Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston; Department of Chemistry, Northeastern University, Boston; and Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD.

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ASSOCIATION OF FIBRIN WITH THE PLATELET CYTOSKELETON

It has been previously postulated that surface membrane proteins become specifically associated with the internal platelet cytoskeleton upon platelet activation. Four lines of evidence support this general hypothesis since the same research group now shows that platelet surface receptors for fibrin become specifically associated with the platelet Triton-insoluble cytoskeleton. (1) Fibrin was detected immunologically in the washed Triton-insoluble cytoskeletons of thrombin-activated platelets under conditions where fibrin polymerization and resultant precipitation was blocked with Gly-Pro-Arg-Pro, a synthetic peptide that inhibits polymerization of fibrin monomer. (2) Radiolabeled fibrin bound to thrombin-activated platelets and became associated with the cytoskeleton. (3) The amount of radiolabeled fibrin bound to thrombin-activated thrombasthenic platelets and their cytoskeletons amounted to about 20% of the fibrin bound to thrombin-activated control platelets and their cytoskeletons. (4) The association of fibrin with cytoskeletons and with the platelet surface was nearly quantitatively blocked by an antibody prepared against cytoskeletons (anti-C), an antibody against isolated membranes of pronase-treated platelets (anti-MI), and a monoclonal antibody to the platelet surface glycoprotein complex, GPII$_b$-GPIII (anti-GPIII). These antibodies blocked ADP and thrombin-induced platelet aggregation as well as thrombin-induced clot retraction. Analysis of the immunoprecipitates obtained with anti-C, anti-MI, and anti-GPIII from detergent extracts of ^{125}I-surface labeled platelets revealed that these antibodies recognized GPII$_b$-GPIII. These data suggest that thrombin activation of platelets results in the specific association of fibrin with the platelet cytoskeleton, that this association may be mediated by the GPII$_b$-GPIII complex, and that these mechanisms may play an important role in platelet aggregation and clot retraction induced by thrombin.

Tuszynski, G. P., Kornecki, E., Cierniewski, C., Knight, L. C., Koshy, A., Srivastava, S., *Niewiarowski, S.*, and Walsh, P. N.

The Journal of Biological Chemistry 259(8):5247-5254, 1984.

Other support: Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

INHIBITION OF FIBRINOGEN RECEPTOR-MEDIATED PLATELET AGGREGATION BY HETEROLOGOUS ANTI-HUMAN PLATELET MEMBRANE ANTIBODY: SIGNIFICANCE OF AN M_r = 66,000 PROTEIN DERIVED GLYCOPROTEIN IIIa

It is generally accepted that the major physiological role of fibrinogen is related to its conversion to fibrin that leads to the formation of blood clots and to the arrest of bleeding. In the study reported here, heterologous anti-human platelet membrane antisera were raised in rabbits against membranes prepared from human intact or chymotrypsin- or pronase-treated platelets. Anti-intact anti-chymotrypsin and anti-pronase-treated platelet membrane antibodies (IgG and Fab fragments) inhibited the fibrinogen-induced aggregation of ADP-stimulated and of chymotrypsin-treated platelets. The specific binding of ^{125}I-fibrinogen to these platelets was also inhibited. As revealed by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ^{125}I-surface-radiolabeled platelet proteins and by the electrophoresis of the immunoprecipi-

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

tates prepared from these labeled proteins, predominant components on the surface of intact platelets were glycoproteins IIb and IIIa. These proteins were immunoprecipitated by all three antibodies. In addition, chymotrypsin-treated platelets contained an $M_r = 66,000$ protein on their surface that was also immunoprecipitated by the three types of anti-platelet membrane antibodies. The appearance of this $M_r = 66,000$ protein on the surface of chymotrypsin-treated platelets correlated with the exposure of fibrinogen receptors on the platelet surface as evidenced by the increased platelet aggregation and the enhanced ^{125}I-fibrinogen binding shown by chymotrypsin-treated platelets.

Other related studies suggested that the $M_r = 66,000$ protein was a proteolytic cleavage product of glycoprotein IIIa. Analysis on sodium dodecyl sulfate gels of the major chymotryptic cleavage products of partially purified glycoprotein IIIa and analysis of the peptide of glycoprotein IIIa immunoprecipitated by anti-pronase-treated platelet membrane antibody revealed that the $M_r = 66,000$ protein produced by chymotrypsin on the platelet surface was the major chymotryptic cleavage product of glycoprotein IIIa. A hypothesis was proposed that the $M_r = 66,000$ is a part of glycoprotein IIIa present on the surface of proteolytically treated platelets and it may function in fibrinogen binding and fibrinogen-induced platelet aggregation and that the 66,000-dalton region of glycoprotein IIIa in intact platelets may represent the fibrinogen-binding domain of glycoprotein IIIa.

Kornecki, E., Tuszynski, G. P., and *Niewiarowski, S.*

The Journal of Biological Chemistry 258(15):9349-9356, 1983.

Other support: National Institutes of Health, National Research Service Award and the Kroc Foundation.

From the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

COMPARISON OF PLATELET FIBRINOGEN RECEPTORS ON INTACT AND PROTEOLYTICALLY-TREATED PLATELETS BY USE OF AN ANTI-GLYCOPROTEIN IIIa MONOCLONAL ANTIBODY (MA 123)

The abstract of this paper notes that a murine monoclonal antibody (MA 123) was selected by screening 153 supernatants of hybridoma cells secreting anti-human platelet antibodies for their ability to inhibit the fibrinogen-induced aggregation of chymotrypsin-treated platelets. MA 123 inhibited the binding of ^{125}I-fibrinogen to ADP-stimulated intact human platelets and to platelets treated with chymotrypsin or pronase. Moreover, it inhibited the fibrinogen-induced aggregation of these platelet suspensions. The degree of inhibition was similar in each of the three types of platelets tested. The interactions of MA 123 with the ^{125}I-labeled surface components of intact and chymotrypsin-treated platelets were studied by immunoprecipitation using *Staphylococcus aureus* coated with goat anti-mouse IgG, followed by SDS-polyacrylamide gel electrophoresis and autoradiography. MA 123 precipitated the glycoprotein IIb-glycoprotein IIIa (GPIIb-GPIIIa) complex from the surface of detergent solubilized intact human platelets; it also precipitated GPIIIa from the surface of chymotrypsin-treated platelets. Partially purified GPIIIa was also immunoprecipitated by MA 123. The data suggest that the exposure of fibrinogen receptors by ADP, chymotrypsin or pronase is associated with alterations of GPIIIa on the platelet surface.

Kornecki, E., Lee,
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Kornecki, E., Lee, H., Merlin, F., Hershock, D., Tuszynski, G. P., and *Niewiarowski, S.*

Thrombosis Research 34:35-49, 1984.

Other support: National Institutes of Health and a Special Investigatorship Award from the American Heart Association, Southeastern Pennsylvania Chapter.

From the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia, and Centre National de Transfusion Sanguine, Paris, France.

SURFACE PROPERTIES OF PULMONARY ENDOTHELIAL CELLS

The authors' long-term goal is to help elucidate the means by which pulmonary endothelial cells participate in establishing the quality of systemic arterial blood. Their special interests are in the cellular and molecular means by which the cells actively process polypeptide hormones, prohormones and other excitatory substances so as to determine which pass on to distant target tissues and which do not. It seems true that the researchers' understanding of pulmonary endothelium as a metabolically active and responsive tissue will depend in large measure on understanding the detailed topography of the endothelial surface in terms of relationships between enzymes, binding sites and extracellular material. Reported here is a method for obtaining surface replicas of endothelial cells showing previously unrecognized structures and the distribution of two known surface enzymes. For these experiments, endothelial cells (bovine pulmonary artery or rabbit pulmonary microvascular) that had never been exposed to proteolytic enzymes were seeded onto glass coverslips in flasks or Lux 8-well dishes. Electron microscope immunocytochemistry and routine transmission electron microscopy were undertaken and assays for angiotensin-converting enzyme and carboxypeptidase N were carried out. From the electron micrographs of surface replicas, results show that it is evident that the endothelial cell surface is far more complex than recognized previously. The new surface replicas show structure on what was previously believed to be ultrastructure. Apparently, the glycocalyx itself occurs as a regular carpet on unreacted cells. Overall, the microstructure, fine structure and ultrastructure of the endothelial cell surface appear well-designed to facilitate interactions between plasma solutes with the cell-bound peptidase enzymes. Using the techniques described here virtually the entire surface of an endothelial monolayer in culture can be replicated and the replicas then examined at the resolution of the transmission electron microscope. The authors believe that this surface replication technique has promise for a wide range of studies, not the least of which are those designed to define at the macromolecular level the precise sites of surface enzymes and possibly hormone receptors.

Ryan, U. S. and Ryan, J. W.

In: *Surface Phenomena in Hemorrheology: Their Theoretical, Experimental and Clinical Aspects*, Annals of the New York Academy of Science 416:441-456, 1983.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

INFLAMMATORY MEDIATORS, CONTRACTION AND ENDOTHELIAL CELLS

Pulmonary endothelial cells possess on their luminal surfaces a number of enzymes capable of processing circulating vasoactive substances. Among these are enzymes that can metabolize putative inflammatory mediators such as the kinins and the anaphylatoxins. The activities of these enzymes presumably serve to protect the systemic circulation from damage by inflammatory mediators and may also act to protect the endothelial cells themselves. In addition to degradative enzymes, the endothelial surface is equipped with receptors for some inflammatory mediators and other excitatory substances, and receptor activation elicits cellular responses. Efforts to elucidate further the cellular and molecular means by which pulmonary endothelial cells process and respond to circulating hormones, mediators and related substances will require understanding not only of the topography of surface enzymes and receptors but also of the intracellular events occurring as part of the integrated cellular response. In the concluding section of this paper, the authors maintain that what they have tried to convey is that structure and function, indeed dysfunction, of endothelium are highly complex. As subthemes, they hope they have made it evident that kinins very likely are not humoral mediators of acute inflammation and this is in spite of their ability, in pharmacologic doses, to increase microvascular permeability and induce a vasodilation in many ways typical of that seen in inflammation. As another subtheme, they hope they have made clear that inhibitors of CPN can make some inflammatory responses very much more severe. Finally, the investigators believe that interactions of endothelium *in vivo* with C1q and, under certain circumstances, C3b and the Fc segment of IgG may well bespeak means by which endothelial cells become active participants in the acute inflammatory response.

Ryan, U. S. and Ryan, J. W.

In: Courtice, F. C., Garlick, D. G. and Perry, M. A. (eds.) *Progress in Microcircular Research*, Sydney: Committee in Postgraduate Medical Education, University of New South Wales, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ENDOTHELIAL SURFACE ENZYMES AND THE DYNAMIC PROCESSING OF PLASMA SUBSTRATES

This article focuses primarily on reactions between endothelial surface peptidase enzymes and circulating oligopeptides. These reactions are fairly well understood and appear to provide new insights into subjects as diverse as blood pressure control and mechanisms of induction of noncardiogenic pulmonary edema. Furthermore, some of the technology developed to clarify understanding of the immediate metabolic fate of angiotensin I and bradykinin appears to have potential for monitoring both the progress of disease states such as adult respiratory distress syndrome and some forms of drug therapy. One section of this paper is devoted to Fine Structure of the Surface Endothelium: (A) Endothelial Projection; (B) Globular Particles Seen by Freeze-Fracture;

(C) Surface Antigens; and
Surface Enzyme *in Vivo*: (
(D) Biochemical Aspects;
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Ryan, J. W. and *Ryan, U.*

International Review of E

Other support: U. S. Pub

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Dotan, I. and *Shechter, I*

Archives of Biochemistry

From the Department of
Aviv University, Tel Avi

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(C) Surface Antigens; and (D) Surface Replicas. A second section covers Assay of Surface Enzyme *in Vivo*: (A) Substrates; (B) Target Enzymes; (C) Assay Procedure; (D) Biochemical Aspects; (E) Specificity and Kinetics; (F) Where Does the Enzyme: Substrate Reaction Occur? (G) Physiological Aspects; and (H) Enzyme Specificity: Effects of Incubation Time. Clinical implications are also considered in this study as well as implications for therapy and patient management. In a concluding section, the authors point out that means for achieving specific pharmacological and immunologic ablations of surface enzymes are implied by previous discussions of enzyme inhibitors and antibodies prepared against some of the enzymes. Judicious treatment of endothelial cells with enzymes capable of catalyzing limited degradations of glycosaminoglycans, proteoglycans, fibronectin, and some forms of collagen may well yield characteristic molecular scars identifiable via surface replicas. Altogether, prospects seem excellent for adding another detailed chapter to current understanding of cellular and molecular physiology and pathology.

Ryan, J. W. and *Ryan, U. S.*

International Review of Experimental Pathology 26:1-43, 1984.

Other support: U. S. Public Health Service and the John A. Hartford Foundation

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

PROPERTIES OF LATENT AND THIOL-ACTIVATED RAT HEPATIC 3-HYDROXYL-3-METHYLGLUTARYL-COENZYME A REDUCTASE AND REGULATION OF ENZYME ACTIVITY

Previous experiments have suggested to these researchers that thiols activate 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) by binding to it. If a protein-disulfide interchange with the activating thiol does occur, it is readily reversible. In the present report, a simple, quick method for the conversion of soluble Ea to E_a and the kinetics of catalysis by various activation states of HMG-CoA reductase are described. Specifically, the effect of the thiols glutathione (GSH), dithiothreitol (DTT) and dithioerythritol (DTE) on the conversion of an inactive, latent form (E_i) of rat liver HMG-CoA reductase to a catalytically active form (E_a) is examined. Latent hepatic microsomal HMG-CoA reductase is activated to a similar degree of activation by DTT and DTE and to a lower extent by GSH. All three thiols affect both K_m and V_{max} values of the enzyme toward HMG-CoA and NADPH. Studies of the effect of DTT on the affinity binding of HMG-CoA reductase to agarose-hexane-HMG-CoA resin shows that thiols are necessary for the binding of the enzyme to the resin. A combination of these and other results presented here points to the importance of the thiol/disulfide ratio for the modulation of hepatic HMG-CoA reductase activity.

Dotan, I. and *Shechter, I.*

Archives of Biochemistry and Biophysics 226(2):401-410, 1983.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

10023191113

Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

REGULATION OF RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

This study was undertaken to elucidate the effect of thiols on the modulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. Specifically, the activation of HMG-CoA reductase by glutathione (GSH) and dithiothreitol (DTT) in rat liver microsomes was studied and compared to the activation by these thiols of enzyme that was solubilized by freeze-thawing. An increase of V_{max} was observed for the two enzyme preparations with increasing concentrations of the two activating thiols. Reactions of GSH-activated microsomal enzyme with increasing concentrations of NADPH show sigmoidal kinetics with a Hill coefficient of 2.01 ±0.07 at 2-4 mM GSH. Increase of the activating GSH concentrations resulted in a gradual change towards Michaelis-Menten kinetics. Activation of the microsomal enzyme by DTT yielded similar results except that a Hill coefficient of 1.1 was observed already at 2.5 mM DTT. Normal Michaelis-Menten kinetics were observed for HMG-CoA at all GSH concentrations. These and other results presented here are compatible with a model in which HMG-CoA reductase activity is GSH-dependent, allosterically modulated under physiological hepatic conditions. In addition, the widely used assay conditions, using high DTT concentrations or employment of highly purified soluble enzyme, precluded the observation of sigmoidal kinetics and the suggested model.

Roitelman, J. and *Shechter, I.*

The Journal of Biological Chemistry 259(2):870-877, 1984.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

MEASUREMENT *IN VIVO* OF IRREVERSIBLE DEGRADATION OF LOW DENSITY LIPOPROTEIN IN THE RABBIT AORTA: PREDOMINANCE OF INTIMAL DEGRADATION

The development of a highly sensitive method for assessing, tissue by tissue, the rates of irreversible protein degradation *in vivo* has allowed the investigators to quantify low density lipoprotein degradation in the normal rabbit aorta and to localize it. The method depends upon the fact that tyramine-cellobiose, like sucrose used in previous studies, can be covalently attached to proteins, enters cells with them, and then remains trapped within the cell after the remainder of the protein molecule has been degraded. Rabbit LDL (d = 1.02 to d = 1.06 g/ml) was labeled with ^{125}I-tyramine-cellobiose and injected into rabbits. Aortic ^{125}I content 24 hours later served as a cumulative measure of degraded LDL (after appropriate corrections for any intact, nondegraded LDL present). Calculated aortic degradation of LDL averaged 9.4×10^{-3} percent of the plasma pool per g aortic wet weight per day (n = 6). Intimal cells, obtained by gentle swabbing, accounted for fully 40% of total aortic degradation even though the intima represented less than 5% of the aortic mass. Autoradiography confirmed the high concentration of label in the intima. Degradation of unmodified and reductively methylated LDL were compared. The fractional rate of degradation of methylated LDL by the intima was 50-60% of that for native LDL, indicating that 40-50% of LDL degradation in the intima, predominantly endothelial cells, is mediated by LDL receptors.

Carew, T. E., Pittman, R. C

Arteriosclerosis 4:214-224, 1

Other support: National He

From the Division of Meta California, San Diego, La J

α-ADRENERGIC-MEDIA SECONDARY TO CAROT CONSCIOUS DOGS

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Murray, P. A., Lavallee, M

Circulation Research 54(1)

Other support: U. S. Public American Heart Associatio

From the Departments of Women's Hospital, Bost Center, Southboro, MA.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

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Carew, T. E., Pittman, R. C., Marchand, E. R., and *Steinberg, D.*

Arteriosclerosis 4:214-224, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego, La Jolla.

α-ADRENERGIC-MEDIATED REDUCTION IN CORONARY BLOOD FLOW SECONDARY TO CAROTID CHEMORECEPTOR REFLEX ACTIVATION IN CONSCIOUS DOGS

The investigators examined the late coronary vascular response to carotid chemoreceptor reflex activation in normal, conscious dogs instrumented for the measurement of right main and left circumflex coronary artery blood flows, arterial and right ventricular pressures, and arterial and coronary sinus blood gases and O_2 contents. With heart rate held constant by electrical stimulation, and with respiration controlled or allowed to vary spontaneously, carotid chemoreceptor reflex activation (induced by intracarotid nicotine) elicited a striking biphasic coronary vascular response characterized by an early dilation (previously described) and a late constriction. For example, with respiration controlled and with the autonomic nervous system intact, carotid chemoreceptor reflex activation resulted in a late increase in arterial pressure (19 ± 4%; $P<0.002$), absolute reductions in right main (24 ± 4%; $P<0.002$), and left circumflex (12 ± 2%; $P<0.004$) coronary blood flows, and increases in right (62 ± 13%; $P<0.002$) and left (26 ± 3%; $P<0.0001$) coronary resistances. This carotid chemoreceptor reflex activation-induced late coronary constriction was also associated with a concomitant increase in myocardial oxygen extraction, i.e., arterial oxygen content remained constant while coronary sinus oxygen content decreased (19 ± 6%; $P<0.04$). Neither propranolol nor atropine had any significant effect on the magnitude of the right coronary constriction. However, both the absolute reduction in right coronary blood flow and increase in right coronary resistance were abolished by phentolamine. Furthermore, either total cardiac denervation or adrenalectomy significantly attenuated ($P<0.01$) carotid chemoreceptor reflex activation-induced reductions in right coronary blood flow and increase in right coronary resistance. We conclude that, with autonomic nervous system activity intact, carotid chemoreceptor reflex activation can elicit an absolute reflexly mediated reduction in coronary blood flow in the normal, conscious dog despite an increase in arterial pressure. The mechanism of the vasoconstriction involves α-adrenergic receptor stimulation mediated by both cardiac sympathetic nerves and circulating catecholamines.

Murray, P. A., Lavallee, M. and *Vatner, S. F.*

Circulation Research 54(1):96-106, 1984.

Other support: U. S. Public Health Service, Massachusetts Heart Association and the American Heart Association.

From the Departments of Medicine, Harvard Medical School, and Brigham and Women's Hospital, Boston, and the New England Regional Primate Research Center, Southboro, MA.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

REFLEX CARDIOVASCULAR RESPONSES TO CHEMORECEPTOR STIMULATION IN CONSCIOUS DOGS WITH CARDIAC HYPERTROPHY

This paper contains the first data to describe the effects of severe RV hypertrophy on reflex cardiovascular responses to carotid chemoreceptor reflex activation (CCRA). α-adrenergic-mediated reflex constriction of the iliac arterial vascular bed in response to CCRA appears normal, whereas the cardiac cycle length response to this reflex stimulus is enhanced in dogs with RV hypertrophy. In striking contrast, an apparent selective abnormality in reflex α-adrenergic vasoconstriction of the coronary vasculature supplying the hypertrophied right ventricle has been documented.

Murray, P. A., and *Vatner, S. F.*

American Journal of Physiology 245(Heart Circ. Physiol.) 14:H871-H879, 1983.

Other support: National Heart, Lung and Blood Institute, Massachusetts Heart Association and the American Heart Association.

From the Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, and the New England Regional Primate Research Center, Southboro, MA.

ALPHA-ADRENERGIC REGULATION OF THE CORONARY CIRCULATION IN THE CONSCIOUS DOG

To examine the role of neural and humoral factors in the control of coronary vasoactivity in conscious animals in which the complicating effects of general anesthesia and recent surgery were absent, dogs were instrumented with miniature pressure gauges in the aorta and left ventricle to measure aortic and left ventricular pressures and with flow probes in the left circumflex coronary artery to measure coronary blood flow. Several weeks after recovery from operation, experiments were conducted in the conscious dogs. Stimulation of the carotid chemoreceptor reflex elicited a biphasic response; initially striking reflex coronary vasodilation was observed. Coronary blood flow tripled even after changes in metabolic factors were minimized by pretreatment wtih propranolol. The chemoreceptor reflex coronary vasodilation was mediated both by cholinergic activation and withdrawal of alpha-adrenergic tone. The second phase of the response involved an increase in coronary vascular resistance, associated with elevated arterial pressure and an absolute reduction in coronary blood flow and coronary sinus oxygen content. The later period of coronary vasoconstriction was abolished by alpha-adrenergic blockade. Intravenous infusion of the alpha-adrenergic agonist methoxamine increased mean arterial pressure by 65 ± 5% and total coronary vascular resistance by 92 ± 14%. To determine whether the large coronary arteries were also under autonomic control, the dogs were instrumented with a pair of ultrasonic crystals on opposing surfaces of the left circumflex coronary artery to measure its diameter directly and continuously. Methoxamine reduced coronary cross-sectional area substantially, thereby constricting large coronary arteries and resistance coronary vessels. Thus, while the coronary arteries are regulated by the changes in myocardial metabolic demands, they are also responsive to changes in neural stimuli. The most striking changes were observed with augmentation and withdrawal of alpha-adrenergic constrictor tone, which appears to be important in the control of large as well as small coronary vessels.

Vatner, S. F.

The American Journal

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From the Departments
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Blood 57(1):106-118, 19

Other support: Nation

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

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Vatner, S. F.

The American Journal of Cardiology 52: 15A-21A, 1983.

U.S. Public Health Service.

From the Departments of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, and the New England Regional Primate Research Center, Southboro, MA.

CONTRIBUTIONS OF HUMAN PLATELETS TO THE PROTEOLYTIC ACTIVATION OF BLOOD COAGULATION FACTORS XII AND XI

Previous studies have suggested that human platelets can promote the activation of factor XI by two different mechanisms, one requiring factor XII and ADP-treated platelets and the other requiring collagen-treated platelets in the apparent absence of factor XII. To investigate these hypotheses, isolated platelets were tested for their capacity to promote the activation and cleavage of purified factors XII and XI in various mixtures of purified factor XII, kallikrein, high molecular weight (HMW) kininogen, and factor XI. The activation of factor XII or factor XI was tested in clotting assays using plasmas deficient in factor XII, XI, IX, or VII and the limited proteolytic cleavage of ^{125}I-labeled factor XII or XI was assessed using sodium dodecyl sulfate polyacrylamide gel electrophoresis in the presence of reducing agents. That ADP- or collagen-treated platelets can promote the proteolytic activation of factor XII in mixtures containing kallikrein and HMW kininogen was shown by: (1) the proteolytic cleavage of factor XII; (2) the development of factor XII, coagulant activity; and (3) the proteolytic cleavage of ^{125}I-factor XI. Platelets treated with collagen or thrombin were shown by both coagulant assays and by cleavage studies to participate with HMW kininogen and kallikrein in the proteolytic activation of Factor XI by mechanisms that were partially dependent upon and partially independent of factor XII. That these zymogen activations are platelet-related was demonstrated by: (1) the enhanced coagulant activity and proteolysis observed after platelet stimulation with ADP, collagen or thrombin; (2) the inhibition of proteolytic activation seen when platelet responses were inhibited by indomethacin; (3) the absence of proteolytic activation in the absence of platelets or other surfaces; and (4) the localization of cleavage products of factor XI in platelet pellets. Experiments with celite or with unstimulated platelets present showed a linear relationship between factor-XII coagulant activity and proteolysis of factor XII. However, with activated platelets present the coagulant activity was two to fourfold greater than that observed for an equivalent amount of factor XII cleaved with celite present. These studies demonstrate that platelets can promote the proteolytic activation of factor XII by kallikrein and of factor XI by both factor-XII dependent and factor-XII-independent mechanisms.

Walsh, P. N. and Griffin, J. H.

Blood 57(1):106-118, 1981.

Other support: National Institutes of Health.

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA, and the Specialized Center on Thrombosis Research, Department of Medicine, Temple University School of Medicine, Philadelphia.

1002319117

Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

NEUTROPHIL-ASSISTED DNA SYNTHESIS BY HUMAN LYMPHOCYTES IN RESPONSE TO MEVALONIC ACID; ENHANCEMENT BY CYTOCHALASIN B

Mevalonic acid, a key intermediate in the biosynthesis of cholesterol, is capable of initiating DNA synthesis, morphologic transformation and cell division in cultures of human peripheral blood lymphocytes. Studies from this laboratory show that pure populations of lymphocytes respond poorly to mevalonic acid, but their response can be enhanced by peripheral blood neutrophils. Addition of cytochalasin B, but not cytochalasin A, to mixed neutrophil-lymphocyte cultures enhances the response of lymphocytes to both Con A and mevalonate, but the increment in mevalonate-induced DNA synthesis far exceeds the enhancement which cytochalasin B produces in the Con A response. As a consequence, the DNA synthetic response in mevalonate containing cells averages 39% of the response to an optimal dose of Con A. These, and several related experiments, suggest that the enhancing effect of cytochalasin B in the response of mixed neutrophil-lymphocyte cultures to mevalonic acid results from its ability to potentiate a time-dependent conditioning effect on lymphocytes which neutrophils exert. To sum up, the ability of mevalonic acid to initiate DNA synthesis and cell cycling in normal and abnormal cells, and the possibility that a nonsterol product of mevalonate metabolism may be the mediator of this effect, adds to the growing knowledge about the relationship between mevalonate metabolism, DNA synthesis and cell replication.

Larson, R. A., Gordon, L. I., and *Yachnin, S.*

Cellular Immunology 81:357-372, 1983.

Other support: U. S. Public Health Service, Nalco Cancer Research Fund, Leukemia Society of America, and the American Cancer Society.

From the Department of Medicine and the Committee on Immunology, the University of Chicago School of Medicine, and the Department of Medicine, Northwestern Medical Center, Chicago.

DIVERGENCE IN CHOLESTEROL BIOSYNTHETIC RATES AND 3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE ACTIVITY AS A CONSEQUENCE OF GRANULOCYTE VERSUS MONOCYTE-MACROPHAGE DIFFERENTIATION IN HL-60 CELLS

The various classes of leukocytes in human peripheral blood differ significantly from each other with respect to cholesterol biosynthesis. In this report, the HL-60 cell line was used to examine and compare the changes in the cholesterol biosynthetic pathway associated with granulocytic or monocyte-macrophage differentiation. Results show that dimethyl sulfoxide-induced granulocyte differentiation is associated with a decrease in cellular 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase activity and with a decrease in the incorporation of [^{14}C] acetate and mevalonate into products of the cholesterol biosynthetic pathway. Phorbol myristate acetate (PMA)-induced monocyte-macrophage differentiation in these cells is associated with a rapid and profound fall in cell proliferation, but nonetheless is accompanied by a dose-dependent increase in cellular HMG-CoA reductase activity and [^{14}C] acetate incorporation into the cholesterol biosynthetic pathway. In addition, PMA induces an increase in [^{14}C] mevalonate incorporation into cholesterol and its precursors, suggesting that post-

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Yachnin, S., Toub, D. S. and

Proceedings of the National
81:894-897, 1984.

Other support: U. S. Public

From the Department of Med
of Chicago, Chicago.

INCREASED 3-HYDROXY
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Yachnin, S. and Mannickar

Blood 63(3):690-693, 1984

Other support: U. S. Publ

From the Department of M
of Chicago, Chicago.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

HMG-CoA reductase events in cholesterol biosynthesis are also enhanced. Mature peripheral blood human monocytes possess an active cholesterol biosynthetic pathway, whereas mature human granulocytes are almost entirely lacking in the ability to synthesize post-squalene products. These investigators' results with HL-60 cells indicate that this divergence in sterol-synthesizing ability between two cell lineages, which normally also derive from a common stem cell, can be observed as an early event in the differentiation process.

Yachnin, S., Toub, D. S., and Mannickarottu, V.

Proceedings of the National Academy of Sciences of the United States of America 81:894-897, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine and the Committee on Immunology, the University of Chicago, Chicago.

INCREASED 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY AND CHOLESTEROL BIOSYNTHESIS IN FRESHLY ISOLATED HAIRY CELL LEUKEMIA CELLS

In this study of cholesterol synthesis, freshly isolated hairy cells from the peripheral blood of patients with hairy cell leukemia (HCL) synthesized 3-5-fold greater amounts of cholesterol, lanosterol and squalene from [1-^{14}C]-acetate that did normal human peripheral blood mononuclear cells under basal conditions of culture (i.e., in the presence of low-density lipoprotein). HCL cells also exhibited an eightfold increase in the activity of the enzyme 3-hydroxyl-3-methylglutaryl coenzyme A reductase. These changes cannot be ascribed to increased rates of cellular proliferation in the HCL cells, nor are they a consequence of an increased rate of loss of newly synthesized cholesterol into the culture medium. The increased rate of cholesterol biosynthesis in HCL cells may result in an increase in their total cellular cholesterol content, as well as in an increase in their plasma membrane cholesterol:phospholipid molar ratio. These changes, in turn, are probably responsible for some of the clinical manifestations of this disease.

Yachnin, S. and Mannickarottu, V.

Blood 63(3):690-693, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine and the Committee on Immunology, the University of Chicago, Chicago.

MEVALONIC ACID INDUCES DNA SYNTHESIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

Recent studies have demonstrated the essential role of mevalonic acid in the regulation of mammalian cell DNA synthesis and cell division and have suggested that a mevalonate-derived nonsterol growth factor may be involved. In the study presented here it was seen that mevalonic acid can stimulate leukemic cells from some patients with B cell chronic lymphocytic leukemia (CLL) to enter the cell cycle *in vitro* and to

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

synthesize DNA. Unlike normal human peripheral blood lymphocytes, which require the presence of granulocytes for a maximal DNA synthetic response to mevalonic acid, CLL cells do not require a helper cell. Only the physiologically active R(−) enantiomer of mevalonic acid is active in initiating DNA synthesis, and no stimulatory effect is noted after addition of the precursors of mevalonic acid or of its known products. The mitogenic responses to mevalonic acid measured in CLL cells from 35 patients varied widely (range of stimulation indices, 1.2-18.6), but the DNA synthetic response of CLL cells from nine patients to mevalonic acid exceeded those seen with such common lymphocyte mitogens as concanavalin A, phytohemagglutinin, pokeweed mitogen, or a phorbol ester. The mevalonate-responsive CLL cell was enriched in the E(−), M(+) rosette subpopulation. When CLL cells were incubated in lipoprotein-depleted serum-containing medium, the presence of low density lipoprotein cholesterol increased their sensitivity to mevalonic acid transformation, suggesting that a nonsterol product of mevalonic acid metabolism may be involved. Mevalonic acid, which is essential for the growth of cells programmed to divide or stimulated to divide by mitogens, can by itself initiate cell replication in relatively inert G_0 phase normal and neoplastic lymphocyte populations.

Larson, R. A. and *Yachnin, S.*

Blood 64(1):257-262, 1984.

Other support: U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Department of Medicine, University of Chicago, Chicago.

IV. *Neuropharmacology and Physiology*

TRANSIENT EXPRESSION OF SELECTED CATECHOLAMINERGIC TRAITS IN CRANIAL SENSORY AND DORSAL ROOT GANGLIA OF THE EMBRYONIC RAT

One outstanding problem in developmental neurobiology concerns the regulation of neuronal phenotypic expression *in vivo*. The present study addresses this problem by describing cellular populations, located in embryonic cranial sensory and dorsal root ganglia, which transiently express catecholamine-specific enzymes *in vivo*. Isolated cells expressing tyrosine hydroxylase (T-OH) immunoreactivity were initially detected in trigeminal (V) ganglion anlages as early as gestation day 10.5 (E10.5; 18-22 somites). Neurofilament (NF) protein was also evident in V at these early stages. By E11.5 (27-30 somites) clusters of T-OH-positive cells were visible in V. Many of these cells were bipolar sending processes into the primitive brainstem. In addition, cells expressing T-OH were apparent in primordia of sensory ganglia serving the glossopharyngeal (IX) and vagal (X) cranial nerves, as well as in rostral dorsal root ganglia. Unlike transiently catecholaminergic cells of the developing intestine, these cells lacked demonstrable catecholamine histofluorescence and a high-affinity uptake process. By this stage (E11.5) all cranial sensory ganglia were rich in NF protein, but immunoreactivity was confined to cellular processes rather than perikarya. By E13.5,

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Jonakait, G. M., Markey, K.

Developmental Biology 101(1

Other support: National Inst

From the Division of Develop
and the Department of Psych

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Andersson, K., *Fuxe, K.*

Acta Physiologica Scandi

Other support: Svenska

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and the Department of H

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

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Cancer Research Fund.

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T-OH was absent from all sensory ganglion neurons. These and other observations suggest that transient expression of neurotransmitters during development is widespread, occurring in cells of diverse origins and fates.

Jonakait, G. M., Markey, K. A., Goldstein, M., and *Black, I. B.*

Developmental Biology 101(1):51-60, 1984.

Other support: National Institutes of Health and the Dysautonomia Foundation.

From the Division of Developmental Neurology, Cornell Medical College, New York, and the Department of Psychiatry, New York University Medical Center, New York.

DIFFERENTIAL EFFECTS OF MECAMYLAMINE ON THE NICOTINE INDUCED CHANGES IN AMINE LEVELS AND TURNOVER IN HYPOTHALAMIC DOPAMINE AND NORADRENALINE NERVE TERMINAL SYSTEMS AND IN THE SECRETION OF ADENOHYPOPHYSEAL HORMONES IN THE CASTRATED FEMALE RAT. EVIDENCE FOR INVOLVEMENT OF CHOLINERGIC NICOTINE-LIKE RECEPTORS

This study evaluated the effects of mecamylamine on the nicotine induced changes in hypothalamic catecholamine (CA) levels and turnover in female rats ovariectomized for one month. In the same group of animals, the serum prolactin, LH, FSH, TSH, GH, and corticosterone levels were measured using radioimmunoassay procedures. Results showed that the nicotine treatment induced a significant depletion of amine stores and an increase of amine turnover in dopamine and noradrenaline (NA) nerve terminals of the median eminance and of the peri- and paraventricular and dorsomedial NA systems of the hypothalamus using the tyrosine hydroxylase inhibition model. Mecamylamine partly counteracted the nicotine induced reduction of amine stores in peri- (anterior part) and paraventricular NA nerve terminal systems as well as the nicotine induced increase of NA turnover in these systems, but not the action of nicotine on the CA systems of the median eminence. Nicotine significantly and markedly reduced prolactin, LH, TSH, and GH secretion and increased corticosterone secretion, but did not influence FSH secretion. These effects were partly counteracted by mecamylamine in the case of prolactin, LH and TSH secretion but not in the case of GH and corticosterone secretion. Overall, the discovered differential blockade of mecamylamine on nicotine induced change in CA levels and turnover in the paraventricular area and the anterior periventricular hypothalamic region as compared with the median eminence region opens up the possibility of the existence of different types of cholinergic nicotine-like receptors with different susceptibility to the blocking activity of mecamylamine and possibly other blockers of cholinergic nicotine-like receptors.

Andersson, K., *Fuxe, K.*, Eneroth, P., and Agnati, L. F.

Acta Physiologica Scandinavica 120:489-494, 1984.

Other support: Svenska Tobaks AB, Stockholm.

From the Department of Histology, Karoklinska Institutet, Stockholm, Hormone Laboratory, Department of Obstetrics and Gynaecology, Karolinska Hospital, Stockholm, and the Department of Human Physiology, University of Modena, Modena, Italy.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

EFFECT OF ETHANOL ON CHOLECYSTOKININ-INDUCED ENZYME SECRETION FROM ISOLATED RAT PANCREATIC ACINI

Cholecystokinin octapeptide (CCK_8)-stimulated amylase release in isolated rat pancreatic acini was inhibited over 30% by 600 mM ethanol. The configuration of the dose-response curve for CCK_8, however, in the presence of the ethanol was similar to that of the control. Amylase release elicited by maximal concentrations of CCK_8 (300 pM) was inhibited by increasing concentrations of ethanol (0.3 to 1.3 M), and this inhibition was concentration dependent. In addition, the binding of [^{125}I]CCK_{33} to specific membrane receptors on acini was inhibited by ethanol in a dose-dependent manner. A positive correlation between the inhibitory effects of ethanol on CCK binding and CCK-induced amylase release was observed. Furthermore, these inhibitory effects of ethanol were reversible. Basal amylase release, however, was increased 20-50% by ethanol between the concentrations of 0.3 and 1.3 M; higher concentrations caused a leakage of amylase from the acini both in the absence and presence of 300 pM CCK_8. This is confirmed by ^{51}Cr release from prelabeled acini which revealed no significant damage to acinar cell membrane between 0.3 and 1.6 ethanol, but significant damage to acini at higher concentrations. These data suggest that the 600 mM ethanol-induced inhibition of CCK action in acini is due to reversible perturbation of the acinar cell membrane.

Lewin, M. B., Sankarae, H., DeVeney, C. W., Wong, A., Wendland, M. F., and *Geokas, M. C.*

Biochemical Pharmacology 33(20):3225-3229, 1984.

Other support: Medical Research Service of the Veterans Administration.

From the Department of Medicine, University of California, Davis; Enzymology Research Laboratory, VA Medical Center, Martinez, CA; and Surgical Service, VA Medical Center and Department of Surgery, University of California, San Francisco.

REDUCTION OF DOPAMINE UPTAKE AND COCAINE BINDING IN MOUSE STRIATUM BY N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyrine (NMPTP) has been shown earlier to have toxic effects on the nigrostriatal dopamine system in the rhesus monkey, with biochemical, histological and behavioral symptoms similar to Parkinsonism. In the study presented here, administration of NMPTP (daily injections of 8 mg/kg for five days via tail vein) reduced [^{3}H]dopamine uptake in striatal synaptosomes by 63% and reduced [^{3}H]cocaine binding to striatal membranes by 61%. [^{3}H]cocaine binding was not affected in olfactory tubercle, suggesting a selective effect of NMPTP on the nigrostriatal but not on the mesolimbic dopaminergic system. The destruction of dopamine terminals in the striatum did not alter (up-regulate) [^{3}H]spiroperidol binding. The present results suggest that NMPTP has degenerative effects on dopaminergic pathways in the nigrostriatal pathway in mice and that it may be useful in developing a rodent model of Parkinson's disease.

Sershen, H., Reith, M. E. A., Hashim, A., and *Lajtha, A.*

European Journal of Pharmacology 102:175-178, 1984.

Other support: National Science Foundation.

From the Center for Neurochemistry, Ward's Island, New York.

FACTORS AFFECTING THE
PLACENTAL VILLUS: ACET
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Placental insufficiency for
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Sastry, B. V. Rama, Barnwell,

Trophoblast Research 1:81-100,

Other support: U.S. Public He

From the Department of Pharm
Nashville, TN.

DEPRESSION OF AMINO AC
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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

FACTORS AFFECTING THE UPTAKE OF α-AMINO ACIDS BY HUMAN PLACENTAL VILLUS: ACETYLCHOLINE, PHOSPHOLIPID METHYLATION, Ca^{++} AND CYTOSKELETAL ORGANIZATION

Placental insufficiency for the transport of amino acids may contribute to intrauterine fetal growth retardation. Therefore, effects of several factors on the uptake of amino acids by isolated placental villus using mainly the nonmetabolizable α-aminoisobutyric acid (AIB) were studied. In a limited number of experiments, aspartic acid, isoleucine and lysine were used. The following results were obtained: (1) 2-Benzoylethyltrimethylammonium (BETA), an inhibitor of choline acetyltransferase (ChA), inhibited AIB uptake, lowered ChA and acetylcholine (ACh) levels and decreased ACh release. (2) BETA inhibited incorporation of metabolizable amino acids into proteins, possibly by lowering ACh levels. (3) α-Naphthoylethyl-trimethylammonium (α-NETA) inhibited ChA and AIB uptake by placental villus. There was a positive relationship between the inhibition of ChA and the depression of AIB uptake by the villous tissue. The inhibition of placental ChA by α-NETA was not reversed by washing the tissue or by dialyzing the homogenates. (4) Placental villus contains two phospholipid N-methyltransferases (PMT I and II). In the presence of S-adenosyl-L-methionine, PMT I converted phosphatidylethanolamine into phosphatidyl-N-methylethanolamine (PME), and PMT II converted PME into phosphatidylcholine. Inhibiting PMTs in plasma membrane by increasing the intracellular levels of S-adenosyl-L-homocysteine (SAH) in the placental villus inhibited AIB uptake. (5) Ca^{++} is required for both release of ACh and AIB uptake. (6) Inhibition of the microtubular assembly by colchicine depressed AIB uptake. (7) Whole placental trophoblast cells are necessary for efficient active uptake of AIB. These observations indicate that (1) ACh synthesis, its release and its activation of the cholinergic receptor; (2) membrane phospholipid methylation, Ca^{++} transport and amino acid transport sytems; and (3) an organized cytoskeletal system are linked to one another for effective transport of AIB across human placental trophoblast.

Sastry, B. V. Rama, Barnwell, S. L. and Moore, R. D.

Trophoblast Research 1:81-100, 1983.

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

DEPRESSION OF AMINO ACID UPTAKE IN HUMAN PLACENTAL VILLUS BY COCAINE, MORPHINE AND NICOTINE

As summarized in this paper, amino acid uptake was measured *in vitro* in intact human placental villus. Active uptake of α-aminoisobutyric acid (AIB), aspartic acid, isoleucine and lysine was demonstrated. The inhibition of the uptake of these amino acids was studied using cocaine, morphine and nicotine. The uptake of AIB was most sensitive to inhibition by these agents at millimolar concentrations, demonstrating that the different amino acid transport systems may be affected differently by these drugs. The metabolism of aspartic acid, isoleucine and lysine in the placenta may be slightly decreased by these drugs. The inhibition of AIB uptake by these drugs was neither simple competitive nor noncompetitive. The uptake of AIB depends on Ca^{++} in the p

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

medium. These findings suggest that the placental cholinergic function, by decreasing ACh release, may affect amino acid uptake.

Barnwell, S. L. and *Sastry, B. V. Rama.*

Trophoblast Research 1:101-120, 1983.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

V. *Pharmacology and Biochemistry*

CLATHRIN LIGHT CHAINS AND SECRETORY VESICLE BINDING PROTEINS ARE DISTINCT

Recently, several groups of investigators have initiated studies on cytosolic proteins that bind to isolated secretory vesicle membranes in the presence of Ca^{++} in order to identify proteins that may regulate exocytosis. Two major chromaffin granule binding proteins, of molecular weights 32,000 (32K) and 34,000 (34K), were reported to have the same mobility on one-dimensional SDS gels as clathrin-associated light chains from the adrenal medulla, and the 34K granule binding protein the same one-dimensional peptide map as the 34K clathrin light chain. These observations support the hypothesis that Ca^{++}-dependent recruitment of soluble light chains to the vesicle membrane may nucleate the assembly of a clathrin coat and initiate endocytosis. However, it is reported here that two-dimensional peptide maps of the clathrin light chains and of all chromaffin granule membrane binding proteins in the 30K range are distinct and, therefore, fail to support this hypothesis. It has also been suggested that some or all of the vesicle binding proteins require calmodulin for their interaction with the membrane. However, it was found that an antagonism of calmodulin by trifluoperazine does not prevent the association of the other cytosolic proteins with the chromaffin granule membrane.

Creutz, C. E. and Harrison, J. R.

Nature 308(5955):208-210, 1984.

Other support: National Science Foundation, the Virginia Affiliate of the American Heart Association, and the University of Virginia Diabetes Research and Training Center.

From the Department of Pharmacology, University of Virginia School of Medicine, Charlottesville.

ON THE CYTOTOXICITY OF SITE-SPECIFIC FENTON MEC

In the work presented here, the phages T2-T7 has been inves bacteriophages are highly suscep even phages are practically resi presence of copper (or iron) ar presence of H_2O_2. Hydrogen pe inactivation and the damage is pr of the copper ions are bound to th histidine fully protect the phage inactivation. OH scavengers suc poly(ethylene glycol) have no pr labeled phages indicate that both result of the exposure to ascorba information as a result of single- contribute to the loss of the pla interpreted in terms of a "site-s binding of the transition metal production of damage. The boun reductants and is subsequently r with vital macromolecules with a "site-specific" formation of OH accounts both for the high damag protect against it.

Samuni, A., Aronovitch, J., Go

European Journal of Biochemis

Other support: U. S. Departme Neuherberg, West Germany.

From the Departments of Mol Chemistry, Hebrew University

MECHANISMS OF THE DISM THE COPPER(II) PHENANTH OF THE COPPER(I) PHENAN AQUEOUS SOLUTION

In view of the role of $(op)_2C$ O_2 in this process, the authors the disproportion of O_2^- and the $(op)_2Cu^{2+}$ by molecular oxygen i technique of pulse radiolysis to phenanthroline $(op)_2Cu^{2+}$) is cap rate constant k_{ox} = (5.1 ± 0.9 $(op)_2Cu^{2+}$ and of the oxidation c

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ON THE CYTOTOXICITY OF VITAMIN C AND METAL IONS: A SITE-SPECIFIC FENTON MECHANISM

In the work presented here, the toxicity of ascorbate towards phage lambda and the phages T2-T7 has been investigated. At room temperature the T-odd and lambda bacteriophages are highly susceptible to ascorbate-induced damage, whereas the T-even phages are practically resistant. The toxicity of ascorbate is dependent on the presence of copper (or iron) and oxygen, although oxygen is not required in the presence of H_2O_2. Hydrogen peroxide is essential for the ascorbate-induced phage inactivation and the damage is prevented by catalase. At the concentrations used, most of the copper ions are bound to the phage particles. Chelating agents such as EDTA or histidine fully protect the phages, whereas salicylate only reduces the rate of phage inactivation. OH scavengers such as sucrose, formate, mannitol, *tert*-butyl alcohol or poly(ethylene glycol) have no protective effect. In addition, experiments with DNA-labeled phages indicate that both phage adsorption and DNA injection are impaired as a result of the exposure to ascorbate and copper. The failure to express the viral genetic information as a result of single- and double-strand breaks in the DNA probably also contribute to the loss of the plaque-forming ability of the phages. These results are interpreted in terms of a "site-specific" Fenton mechanism according to which the binding of the transition metal ions to the biological target is a prerequisite for the production of damage. The bound metal ion is reduced either by O_2^-, ascorbate or other reductants and is subsequently reoxidized by H_2O_2 yielding OH^- radicals which react with vital macromolecules with a high probability of causing "multi-hit" damage. This "site-specific" formation of OH^- radicals, which takes place near the target molecules, accounts both for the high damaging efficiency and for the failure of OH^- scavengers to protect against it.

Samuni, A., Aronovitch, J., Godinger, D., Chevion, M., and *Czapski, G.*

European Journal of Biochemistry 137:119-124, 1983.

Other support: U. S. Department of Energy and Gesellschaft für Strahlenforschung Neuherberg, West Germany.

From the Departments of Molecular Biology, Cellular Biochemistry and Physical Chemistry, Hebrew University of Jerusalem, Israel.

MECHANISMS OF THE DISMUTATION OF SUPEROXIDE CATALYZED BY THE COPPER(II) PHENANTHROLINE COMPLEX AND OF THE OXIDATION OF THE COPPER(I) PHENANTHROLINE COMPLEX BY OXYGEN IN AQUEOUS SOLUTION

In view of the role of $(op)_2Cu^{2+}$ in the degradation of DNA, and the possible role of O_2^- in this process, the authors decided to study the catalytic effect of $(op)_2Cu^{2+}$ upon the disproportion of O_2^- and the kinetics and the reaction mechanism of the oxidation of $(op)_2Cu^{2+}$ by molecular oxygen in the presence and in the absence of H_2O_2. By using the technique of pulse radiolysis to generate O_2^-, it is demonstrated here that copper 1,10-phenanthroline $(op)_2Cu^{2+}$ is capable of catalytically dismutating O_2^- with a "turnover" rate constant $k_{cat} = (5.1 \pm 0.9) \times 10^8\ M^{-1}\ s^{-1}$. The rate constants of the reduction of $(op)_2Cu^{2+}$ and of the oxidation of $(op)_2Cu^{2+}$ by O_2^- have been determined to be (1.93 ±

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

0.07) × 10^8 and (2.95 ± 0.3) × 10^8 M^{-1} s^{-1}, respectively. The kinetic results of the oxidation of (op)$_2Cu^{2+}$ by molecular oxygen in aqueous solution are interpreted by a mechanism that proceeds via a superoxide intermediate, and the rate constant k-10 = (5.0 ± 0.3) × 10^4 M^{-1} s^{-1} has been determined. The rate constant of the oxidation of (op)$_2Cu^{2+}$ by H_2O_2 was measured to be k_{32} = (937 ± 20) M^{-1} s^{-1}.

Goldstein, S. and *Czapski, G.*

Journal of the American Chemical Society 105(25):7276-7280, 1983.

Other support: U. S. Department of Energy.

From the Department of Physical Chemistry, the Hebrew University of Jerusalem, Jerusalem, Israel.

ROLES OF COPPER AND O_2^- IN THE RADIATION-INDUCED INACTIVATION OF T7 BACTERIOPHAGE

As reported in this paper, the effect of copper on the radiation damage induced in T7 bacteriophage has been investigated. The phages were α-irradiated and the effects of copper(II) ions in the presence of various additives and radical scavengers were examined in an attempt to better understand the effect of transition metal ions on the role of free radicals, particularly superoxide, in biological damage. This work extends a study previously done on isolated enzyme to a whole biological entity. Copper(II) ions, even at very low concentrations, enhanced the lethal effect of radiation. This sensitization was observed in both the presence and absence of oxygen. The effect of copper could be reverted by chelating agents such as EDTA or 1,10-phenanthroline. Hydrogen peroxide enhanced the sensitizing effect of copper, though little if any protection was provided by catalase or SOD. High molecular weight scavengers of free radicals in the presence of both copper(II) and hydrogen peroxide had no protective effect. (This is in contrast to metal-free systems where, although such scavengers are incapable of penetrating the phages, they protect them against inactivation.) Low molecular weight scavengers of free radicals reduced but did not eliminate the sensitizing effect of copper. The sensitizing effect was also observed with other T-odd phages, but not with the T-even series. The results are interpreted in terms of a site-specific Fenton mechanism according to which the binding of the metal ion to the phages is a prerequisite for the occurrence of the biological damage. The results also indicate that most of the copper effect is *endogenous*. This is in accord with the failure of copper to sensitize the T-even phages, which differ by the rigidity and permeability of their outer coat structures.

Samuni, A., Chevion, M. and *Czapski, G.*

Radiation Research 99:562-572, 1984.

Other support: United States-Israel Binational Science Foundation and the Gesellschaft fur Strahlenforschung Neuherberg, West Germany.

From the Department of Molecular Biology, Department of Cellular Biochemistry, and Department of Physical Chemistry, the Hebrew University of Jerusalem, Israel.

AN EPR DATA SYSTEM E
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McLaughlin, A. et al.

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Other support: National Insti

From the Biology Department
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of New York, Stony Brook.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

AN EPR DATA SYSTEM BASED ON S-100 BUS HOME COMPUTER COMPONENTS

This article presents a technique for construction of a laboratory data system using commercially available S-100 (IEEE-696) bus components. The system was designed to work specifically with an IBM/Bruker ESR spectrometer, but the design is flexible enough that the system could easily be used with a variety of laboratory instruments. The software package which was written for this system allows the user to acquire either analog or digital data from a laboratory instrument, signal average the analog data and store the data on disk files. The S-100 bus components are inexpensive and one is able to construct a complete data system at minimum cost. A final part of this article describes software which has been developed for processing EPR data.

Schultz, R., Hurst, G., Thieret, T. E., and *Kreilick, R. W.*

Journal of Magnetic Resonance 53:303-312, 1983.

Other support: National Institutes of Health.

From the Department of Chemistry, University of Rochester, Rochester, NY.

DIMETHONIUM, A DIVALENT CATION THAT EXERTS ONLY A SCREENING EFFECT ON THE ELECTROSTATIC POTENTIAL ADJACENT TO NEGATIVELY CHARGED PHOSPHOLIPID BILAYER MEMBRANES

The summary of the paper presented here states that calcium and other alkaline earth cations change the electrostatic potential adjacent to negatively charged bilayer membranes both by accumulating in the aqueous diffuse double layer adjacent to the membrane and by adsorbing to the phospholipids. The effects of these cations on the electrostatic potential are described adequately by the Gouy-Chapman-Stern theory. The investigators report the results of experiments with ethane-bis-trimethylammonium, a cation that has been termed "dimethonium" or "ethamethonium" in analogy with hexamethonium (hexane-1,6-bis-trimethylammonium) and decamethonium (decane-1,10-bis-trimethylammonium). They examined the effect of dimethonium on the zeta potential of multilammellar vesicles formed from the negative lipid phosphatidylserine (PS) and from 5:1 phosphatidylcholine/phosphatidylserine mixtures in solutions containing 0.1, 0.01 and 0.001 M sodium, cesium, or tetramethylammonium chloride. The researchers also examined the effect of dimethonium on the conductance of planar PS bilayer membranes and the ^{31}P NMR signal from sonicated PS vesicles formed in 0.1 M NaCl. They found no evidence that dimethonium adsorbs specifically to bilayer membranes. All the results, except for those obtained with vesicles of low charge density formed in a solution with a high salt concentration, are consistent with the predictions of the Gouy-Chapman theory. The investigators conclude that dimethonium, which does not have the pharmacological effects of hexamethonium and decamethonium, is a useful divalent cation for physiologists interested in investigating electrostatic potentials adjacent to biological membranes.

McLaughlin, A. et al.

The Journal of Membrane Biology 76:183-193, 1983.

Other support: National Institutes of Health and the National Science Foundation.

From the Biology Department, Brookhaven National Laboratory, Upton, NY, and the Department of Physiology and Biophysics, Health Sciences Center, State University of New York, Stony Brook.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

LARGE DIVALENT CATIONS AND ELECTROSTATIC POTENTIALS ADJACENT TO MEMBRANES: EXPERIMENTAL RESULTS WITH HEXAMETHONIUM

As presented here, a simple extension of the Gouy-Chapman theory predicts that the ability of a divalent cation to screen charges at a membrane-solution interface decreases significantly if the distance between the charges on the cation is comparable with the Debye length. The researchers tested this prediction by investigating the effect of hexamethonium on the electrostatic potential adjacent to negatively charged phospholipid bilayer membranes. The distance between the two charges of an extended hexamethonium molecule is ~1nm, which is the Debye length in the 0.1 M monovalent salt solutions used in these experiments. Six different experimental approaches were utilized. The investigators measured the electrophoretic mobility of multilamellar vesicles to determine the zeta potential, the line width of the ^{31}P nuclear magnetic resonance (NMR) signal from sonicated vesicles to calculate the change in potential at the phosphodiester moiety of the lipid, and the conductance of planar bilayer membranes exposed to either carriers (nonactin) or pore formers (gramicidin) to estimate the change in potential within the membrane. They also measured directly the effect of hexamethonium on the potential above a monolayer formed from negative lipids, and attempted to calculate the change in the surface potential of a bilayer membrane from capacitance measurements. With the exception of the capacitance calculations, each of the techniques gave comparable results: hexamethonium exerts a smaller effect on the potential than that predicted by the classic screening theory. The results are consistent with the predictions of the extended Gouy-Chapman theory and are relevant to the interpretation of physiological and pharmacological experiments that utilize hexamethonium and other large divalent cations.

Alvarez, O., Brodwick, M., Latorre, R., *McLaughlin, A.*, McLaughlin, S., and Szabo, G.

Biophysical Journal 44:333-342, 1983.

Other support: University of Chile, National Institutes of Health and the National Science Foundation.

From the Departamento de Biologica, Facultad de Ciencias Básicas Farmacéuticas, Universidad de Chile, Santiago; Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston; Department of Physiology and Biophysics, Harvard Medical School, Boston; Biology Department, Brookhaven National Laboratory, Upton, NY; and Department of Physiology and Biophysics, Health Sciences Center, State University of New York, Stony Brook.

INCREASED SISTER-CHROMATID EXCHANGE IN BONE MARROW CELLS OF MICE EXPOSED TO WHOLE CIGARETTE SMOKE

Previous studies have shown that exposure of BC3F1/Cum female mice to whole cigarette smoke resulted in the induction of the pulmonary macrosomal enzymes, aryl hydrocarbon hydroxylase, ethoxyresorufin-*O*-deethylase, and ornithine decarboxylase. In the study presented here, using defined cigarette smoke exposure conditions, BC3F1/Cum mice were exposed nose-only to two different types of whole cigarette

smoke on a daily basis for or
chromatid exchanges (SCEs) p
Studies were scheduled so that
the last smoke exposure. Expos
or up to 46 weeks resulted in
mice. In animals exposed eithe
the increase in SCEs persisted
This is the first demonstration
been exposed to cigarette smo

Benedict, W. F. *et al.* (*Micro*

Mutation Research 136:73-80

From the Division of Hemat
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fornia School of Medicine, L
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Mitchell, J. A. and Hamn

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smoke on a daily basis for one week and up to 46 weeks. The number of sister-chromatid exchanges (SCEs) per metaphase was determined in bone-marrow cells. Studies were scheduled so that all cytogenetic observations were made 2-3 days after the last smoke exposure. Exposure to either type of smoke on a daily basis for one week or up to 46 weeks resulted in a 2-fold increase in SCEs over sham-exposed control mice. In animals exposed either chronically or for one week to either type of smoke, the increase in SCEs persisted for at least one week after cessation of smoke exposure. This is the first demonstration of the induction of SCEs in laboratory animals that have been exposed to cigarette smoke *in vivo*.

Benedict, W. F. *et al.* (*Microbiological Associates*)

Mutation Research 136:73-80, 1984.

From the Division of Hematology-Oncology, Department of Medicine, Children's Hospital of Los Angeles, and Department of Pediatrics, University of Southern California School of Medicine, Los Angeles; and Division of Toxicology and Oncology, Microbiological Associates, Bethesda, MD.

EIN VERGLEICH DER WIRKUNGEN VON NIKOTIN UND SEROTONIN AUF PERIIMPLANTATIONSPHÄNOMENE IN DER RATTE

In previous investigations we established that the vasoactive substances nicotine and serotonin clearly have distinguishable actions on the course of early pregnancy in rats: nicotine reduced the growth of the blastocyst, delayed the loss of the zona pellucida and retarded implantation without impairing fertility. In contrast, serotonin destroyed the integrity of the implantation site and was embryotoxic. Since prior to implantation the fertilized egg is free living in the lumen of the reproductive tract and is, therefore, fully dependent on diffusion for its nourishment and the exchange of metabolic products, the blastocyst is highly susceptible to changes in the intraluminal environment. The following experiments were carried out to compare the actions of nicotine and serotonin on uterine blood flow and on intrauterine oxygen tension. The actions of a single subcutaneous injection of 5 mg/kg nicotine, 20 mg/kg serotonin or saline (as control) on blood flow at the implantation site of pregnant rats on Day 5 of pregnancy and on oxygen tension on Day 4 of pseudopregnancy were determined. Blood flow was measured with ^{86}Rb (rubidium); intraluminal pO_2 was determined polarimetrically. Results indicate that while the actions of both nicotine and serotonin on implantation site blood flow and intrauterine pO_2 were comparable both in degree and duration, their actions on blastocyst survival were clearly different. It is concluded that blastocysts can survive protracted oxygen deficiency following serotonin or nicotine-induced vasoconstriction. Thus, the embryotoxic effects of serotonin result from some action(s) of the amine other than reduced implantation site blood flow and concomitant intrauterine hypoxia.

Mitchell, J. A. and Hammer, R. E.

Verhandlungen der anatomischen Gesellschaft 77:425-426, 1983.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

QUANTIFICATION OF GUANYLATE CYCLASE CONCENTRATIONS BY A DIRECT DOUBLE DETERMINANT TANDEM IMMUNORADIOMETRIC ASSAY

In this paper, the authors have described the development and application of a simple and direct tandem immunoradiometric assay for quantitating guanylate cyclase protein, independent of enzyme activity, in crude samples. Since the assay uses two antiguanylate cyclase monoclonal antibodies directed to different determinants on the protein, it is a very specific assay. To be exact, a total of 16 monoclonal antibodies have been produced to soluble guanylate cyclase. Two of the antibodies, designated H_6 (IgG_{2a} subclass) and B_4 (IgG_1 subclass), were iodinated and the characteristics of their binding to immobilized guanylate cyclase were examined. Scatchard transformations of binding data were noted. Competitive binding studies revealed that antibodies H_6 and B_4 recognize different determinants on the enzyme. The two antibodies were used to develop a direct, double determinant tandem immunoradiometric assay for soluble guanylate cyclase based on the differential binding of mouse immunoglobulin subclasses to Protein A on *S. aureus* membranes. The investigators also used this assay to quantitate soluble guanylate cyclase protein, independent of enzymatic activity, in a variety of rat tissues. These studies showed lung to be a rich source of the enzyme. Measurements of guanylate cyclase protein were not altered by agents that activate (sodium nitroprusside) or inactivate (cystamine) the enzyme. The ability to measure guanylate cyclase protein, independently of catalytic activity or cyclic GMP levels, should prove extremely useful in studying the function and regulation of this enzyme-nucleotide system.

Lewicki, J. A., Chang, B. and *Murad, F.*

The Journal of Biological Chemistry 258(6):3509-3515, 1983.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Stanford University, and Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

PARTIAL PURIFICATION AND CHARACTERIZATION OF PARTICULATE GUANYLATE CYCLASE FROM RAT LIVER AFTER SOLUBILIZATION WITH TRYPSIN

As summarized here, guanylate cyclase from 105,000 × g particulate fractions of rat liver homogenates (20 pmoles of cyclic GMP formed/min/mg protein) was solubilized in the absence of detergents by incubating fractions 12 min at 37° with 5 ug/ml trypsin. Optimal solubilization was dependent upon trypsin and particulate preparation concentrations. Virtually no activation of particulate guanylate cyclase was observed at any time point or trypsin concentration tested. Guanylate cyclase solubilized with trypsin was purified about 500-fold (9.4 nmoles/min/mg protein) using ammonium sulfate precipitation, GTP-affinity chromatography, and preparative polyacrylamide gel electrophoresis. Activity eluted as a single peak on Sephadex G-200 (Stokes radius = 40Å) and migrated as a single peak on sucrose density gradients ($S_{20,w}$ = 4.6). Thus, the tryptic fragment was estimated to be about 80,000 daltons (Mr) with a frictional ratio (f/f_0) of 1.4. These partially purified preparations exhibited linear double reciprocal plots with Mn-GTP and Hill coefficients of 1.0. This is in contrast to the crude membrane-associated enzyme which had a Hill coefficient of 1.5. These and other

studies indicate that particulate gua
amenable to purification by "clas
purified fragment contains the cata
least one sulfhydryl group required

Waldman, S. A., Lewicki, J. A.,

Journal of Cyclic Nucleotide Rese

Other support: National Institutes

From the Departments of Medicin
cology, Stanford University Veter

EFFECT OF OUABAIN AND A
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procedure which inhibits the Na^+
of sodium nitroprusside on relaxa
induced accumulation of cyclic G
may induce relaxation through c
and or hyperpolarization of the

Rapoport, R. M. and *Murad, F.*

Blood Vessels 20:255-264, 1983

Other support: National Institut

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studies indicate that particulate guanylate cyclase solubilized by limited proteolysis is amenable to purification by "classical" chromatographic techniques. The partially purified fragment contains the catalytic site, the site for nitric oxide activation, and at least one sulfhydryl group required for activity.

Waldman, S. A., Lewicki, J. A., Brandwein, H. J., and *Murad, F.*

Journal of Cyclic Nucleotide Research 8(6):359-370, 1982.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Division of Clinical Pharmacology, Stanford University Veterans Administration Hospital, Palo Alto, CA.

EFFECT OF OUABAIN AND ALTERATIONS IN POTASSIUM CONCENTRATION ON RELAXATION INDUCED BY SODIUM NITROPRUSSIDE

The purpose of the present study was to investigate (1) whether relaxation induced by nitroprusside is effected by agents and procedures known to alter the activity of the Na^+, K^+-pump, and (2) if relaxation to nitroprusside was effected, whether the changes induced by nitroprusside could be mediated through the formation of cyclic GMP. Results of this study show that relaxation of the rat thoracic aorta induced by sodium nitroprusside, 8-bromo-cyclic GMP and cyclic nucleotide phosphodiesterase inhibitor M & B 22,948 was inhibited by exposure to K^+-free solution and ouabain in a concentration-dependent manner. Relaxation occuring with the change in potassium concentration in media from 1 to 2 to 10 mM was increased by sodium nitroprusside, 8-bromo-cyclic GMP and M & B 22,948. Thus, agents and procedures known to decrease and increase the activity of the Na^+, K^+-pump, and presumably alter the membrane potential, inhibited and enhanced relaxation, respectively. Exposure to 1 mM K^+ solution increased the relaxation to low concentrations of sodium nitroprusside, but had no effect on relaxation induced by 8-bromo-cyclic GMP or M & B 22,948. Thus, another procedure which inhibits the Na^+, K^+-pump enhanced the effect of low concentrations of sodium nitroprusside on relaxation. Ouabain had no effect on sodium nitroprusside-induced accumulation of cyclic GMP. These results suggest that sodium nitroprusside may induce relaxation through cyclic GMP formation, effects on the Na^+, K^+-pump and/or hyperpolarization of the smooth muscle cell membrane.

Rapoport, R. M. and *Murad, F.*

Blood Vessels 20:255-264, 1983.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine and Veterans Administration Medical Center, Palo Alto, CA.

AGONIST-INDUCED ENDOTHELIUM-DEPENDENT RELAXATION IN RAT THORACIC AORTA MAY BE MEDIATED THROUGH cGMP

The purpose of this study was to test the hypothesis that relaxation of vascular smooth muscle with other agents that are dependent upon the endothelium, may also be

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mediated through the formation of cGMP within the smooth muscle. Results showed that relaxation of the rat thoracic aorta to acetylcholine, histamine, and Ca^{++} ionophore A23187 was associated with increased levels of cGMP in a time- and concentration-dependent manner, whereas cAMP levels were unaltered. Removal of the endothelium prevented relaxation to these agents and prevented the increased levels of cGMP. Removal of the endothelium after exposure to acetylcholine only partially decreased the elevated levels of cGMP, suggesting that the changes in cGMP occurred within the smooth muscle cells. Eicosatetraenoic acid, an inhibitor of lipoxygenase and cyclooxygenase, and quinacrine, an inhibitor of phospholipase, prevented and reversed acetylcholine-induced relaxation, respectively, and inhibited acetylcholine-induced increased levels of cGMP. In contrast, sodium nitroprusside-induced relaxation and increased levels of cGMP were independent of the presence of the endothelium, exposure to eicosatetranoic acid and quinacrine. The present results support the hypothesis that vascular smooth muscle relaxation induced by some agents is dependent on the presence of the endothelium and is mediated through the formation of an endothelial factor that increases cGMP levels in smooth muscle.

Rapoport, R. M. and *Murad, F.*

Circulation Research 52:352-357, 1983.

Other support: National Institutes of Health, Veterans Administration and a National Research Service Award.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

THE EFFECT OF SOLVENT POLARITY UPON ROTATIONAL BARRIERS IN NIKETHAMIDE

This is a report on a chemical property of nikethamide which could account, in part, for its widespread action on the central nervous system: the hindered internal rotation of the diethylamide group. In summary, dynamic nuclear magnetic resonance techniques were used to study the hindered internal rotation of the amide bond of the analeptic nikethamide. The rotatory motion of this bond was studied in a series of solvents of increasing polarity: $CDCl_3$, $CH_3(CH_2)_4OD$, CH_3CH_2OD, CH_3OD and D_2O. Motion about the amide bond was increasingly hindered in direct proportion to solvent polarity, correlating with enhanced hydrogen bond formation between nikethamide and the more polar solvent molecules. Diethylamide group motion would be expected to affect binding of the carbonyl oxygen to cholinergic receptor sites. The degree to which association to a receptor site can be affected by this rotatory motion may vary from 0 to 4 kcal/mole, the variability being entirely dependent upon the polarity of the binding site. An increase in rotamer lifetime, corresponding to a more polar environment, would be expected to enhance the kinetics of nikethamide association to the receptor site.

Bean, J. W. and *Nelson, D. J.*

Biochemical Pharmacology 33(13):2145-2149, 1984.

From the Chemistry Department, Clark University, Worcester, MA.

^{155}Eu AS A PROBE OF CHOLIN ACETYLCHOLINE RECEPTOI *MELANOGASTER* AND *TORPI*

For this study, $^{155}Eu^{3+}$ exch receptor proteins isolated from t cerebral ganglion of *Drosophila n* nuclear counting apparatus desig apparatus continuously monitors of two flow dialysis cells facilitati protein is dialyzed against buffer perturbed by the presence of nicc $^{155}Eu^{3+}$ displacement from the re related to the structure of the lig tonated pyridyl nitrogen atoms ir tonated forms of the molecules monium ion and carbamylcholi displaces bound $^{155}Eu^{3+}$ from the tetraethylammonium ion does, v choline does.

Bean, J. W., Rosenthal, L. S.,

Journal of the Less-Common M

From the Department of Chemi ter, MA; Department of Pharma Worcester.

ESR SPIN-TRAPPING STUD NOx/OLEFIN REACTIONS: OF THE APPARENTLY LON CIGARETTE SMOKE

Gas-phase cigarette smok carbon-centered free radicals. several variations of the electror use of spin traps in the solid stat gas phase. These gas-phase rad more than 5 min old, a result th of oxygen- and carbon-centere authors hypothesize that free cigarette smoke and exist in a *st* radicals can be formed involve oxide (which acts as a "radical Nitrogen dioxide can then react to produce the radicals that they the reactions of NO/air mixture most abundant species in smok

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^{153}Eu AS A PROBE OF CHOLINERGIC LIGAND INTERACTIONS WITH ACETYLCHOLINE RECEPTOR PROTEINS ISOLATED FROM *DROSOPHILA MELANOGASTER* AND *TORPEDO CALIFORNICA*

For this study, $^{153}Eu^{3+}$ exchange experiments were performed on acetylcholine receptor proteins isolated from the electroplax tissue of *Torpedo californica* and the cerebral ganglion of *Drosophila melanogaster* utilizing a dual-chambered flow dialysis nuclear counting apparatus designed and constructed in the authors' laboratory. The apparatus continuously monitors $^{153}Eu^{3+}$ γ ray emission from the protein compartments of two flow dialysis cells facilitating the measurement of exchange half-lives. Receptor protein is dialyzed against buffer in the first cell, while in the second cell the receptor is perturbed by the presence of nicotinic ligand in the dialysate. Nicotinic ligands induce $^{153}Eu^{3+}$ displacement from the receptor proteins of both species in a manner directly related to the structure of the ligand. Nicotine and nikethamide molecules with protonated pyridyl nitrogen atoms induce $^{153}Eu^{3+}$ exchange significantly, while the deprotonated forms of the molecules do not effect exchange. Acetylcholine, tetraethylammonium ion and carbamylcholine all possess a quarternary nitrogen. Acetylcholine displaces bound $^{153}Eu^{3+}$ from the acetylcholine receptor proteins more readily than the tetraethylammonium ion does, which in turn induces exchange better than carbamylcholine does.

Bean, J. W., Rosenthal, L. S., *Nelson, D. J.*, and Wright, G. E.

Journal of the Less-Common Metals 94:367-374, 1983.

From the Department of Chemistry, Jeppson Laboratory, Clark University, Worcester, MA; Department of Pharmacology, University of Massachusetts Medical Center, Worcester.

ESR SPIN-TRAPPING STUDY OF THE RADICALS PRODUCED IN NOx/OLEFIN REACTIONS: A MECHANISM FOR THE PRODUCTION OF THE APPARENTLY LONG-LIVED RADICALS IN GAS-PHASE CIGARETTE SMOKE

Gas-phase cigarette smoke contains high concentratons of both oxygen- and carbon-centered free radicals. The investigators have detected these radicals using several variations of the electron spin resonance spin-trapping technique, including the use of spin traps in the solid state, to show that the radicals are trapped *directly* from the gas phase. These gas-phase radicals can still be trapped from gas-phase smoke that is more than 5 min old, a result that is clearly inconsistent with the highly reactive nature of oxygen- and carbon-centered radicals. To rationalize this apparent paradox, the authors hypothesize that free radicals are continuously produced and destroyed in cigarette smoke and exist in a *steady* state. They suggest that one mechanism by which radicals can be formed involves the slow oxidation of the relatively unreactive nitric oxide (which acts as a "radical reservoir") to the much more reactive nitrogen dioxide. Nitrogen dioxide can then react with a number of the species that are present in smoke to produce the radicals that they detect. The model used by the researchers consisted of the reactions of NO/air mixtures with unsaturated hydrocarbons. Isoprene is one of the most abundant species in smoke and is known to be very reactive toward NO_x; there-

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fore, they have studied the nature of the radicals that can be spin trapped from gaseous mixtures of NO_2, isoprene and air. They found that the NO/air/isoprene model system gives essentially the same types of radicals (oxygen- and carbon-centered) as does cigarette smoke. They have also studied the gas-phase reactions of NO_2 with several small olefins and 1,3-butadiene and found evidence for peroxyl radical intermediates. In solution, NO_2 reacts with isoprene much faster than it does with the spin-trap phenyl-*tert*-butylnitrone (PBN). The researchers find that NO_2 oxidizes PBN to benzoyl *tert*-butyl nitroxide and propose a mechanism for this reaction.

Pryor, W. A., Tamura, M., and Church, D. F.

Journal of the American Chemical Society 106(18):5073-5079, 1984.

Other support: National Institutes of Health.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

NICOTINE AND PROSTAGLANDIN BIOFORMATION: AN *IN VITRO* STUDY WITH SPECIAL REFERENCE TO SOME TISSUES IN THE VASCULAR SYSTEM IN ANIMALS AND IN MAN

The effect of nicotine on the conversion of arachidonic acid (AA) to prostacyclin (PGI_2), primary prostaglandins (PGs) and thromboxane (Tx) in the rabbit, guinea pig, rat, and human vascular system *in vitro* was investigated. The effect of nicotine was also studied on a purified enzyme — prostaglandin endoperoxide synthetase (PES) — prepared from ram seminal vesicles. Results showed that prostacyclin production by aortic tissue from rabbit, guinea pig and rat — either spontaneous or following incubation with ^{14}C-AA — was inhibited dose-dependently by nicotine. The inhibition was competitive and localized to the enzyme, PES, that converts AA to PG endoperoxide. Nicotine did not affect the enzyme PGI_2 synthetase. It was found that in all species investigated, nicotine dose-dependently inhibited the formation of primary PGs by renal homogenates or microsomal fractions. The formation of TxB_2, in which the two enzymes PES and Tx synthetase are involved, in incubations of platelets with AA was completely unaffected by nicotine in all species. Also, experiments with the purified enzyme PES confirmed the inhibitory effect of nicotine. Overall, the data clearly demonstrate that nicotine inhibits PES in some but not all tissues in the vascular system in the investigated animals and in man. The possible implication of such a tissue difference in sensitivity to nicotine, for the relation between tobacco smoking and cardiovascular disease, is pointed out.

Alster, P. (*Wennmalm, Å.*)

Thesis, Stockholm, 1983.

Other support: The Swedish Tobacco Company, the Swedish Medical Research Council, Karolinska Institutet, and Stiftelsen Clas Groschinskys Minnesfond.

From the Department of Clinical Physiology, Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden.

EFFECT OF NICOTINE ON TH ACTIVITY AND THROMBOX

The effect of nicotine on the ane (TxB_2) in rabbit aorta and pla ment, rabbit aortic rings were inc incubation products were separ ternatively, the aortic rings were formation of platelet anti-aggreg somes were incubated with [^{14}C, t.l.c. In addition, rings of aorta w AA to labeled 6-keto-$PGF_{1\alpha}$ (the incubated in saline medium spon was dose-dependently inhibited b that platelet microsomes converte fected by nicotine. In summary, nicotine exists between cycloox demonstrate a tissue difference utilization of exogenous AA as su

Alster, P. and *Wennmalm, Å.*

British Journal of Pharmacology

Other support: Swedish Tobacco fond.

From the Department of Clinica dinge, Sweden.

VI. *Immunolog*

SUPPRESSOR T CELL ACTIV FERON

The interferons are a group c variety of immune responses. M suppressor T lymphocytes *in vitr* spleen cells. Suppression of huma here to determine whether human cyte IFN (IFNα) suppressed po ulin production by human periph doses of 200 to 350 U/ml. Resp dose-dependent manner; control studies, PBMC incubated with I cells that decreased pokeweed m duction by autologous cells by prevented by catalase, ascorbic

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pin trapped from gaseous
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with the spin-trap phenyl-
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79, 1984.

uisiana State University.

AN *IN VITRO* STUDY
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acid (AA) to prostacyclin
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EFFECT OF NICOTINE ON THE FORMATION OF PROSTACYCLIN-LIKE ACTIVITY AND THROMBOXANE IN RABBIT AORTA AND PLATELETS

The effect of nicotine on the bioformation of prostacyclin (PGI_2) and of thromboxane ($Tx)B_2$ in rabbit aorta and platelets, respectively, was investigated. In one experiment, rabbit aortic rings were incubated with [^{14}C]-arachidonic acid ([^{14}C]-AA) and the incubation products were separated with thin layer chromatography (t.l.c.). Alternatively, the aortic rings were incubated without substrate and their spontaneous formation of platelet anti-aggregatory activity was measured. Rabbit platelet microsomes were incubated with [^{14}C]-AA and the products formed were separated with t.l.c. In addition, rings of aorta were found to be incapable of converting added [^{14}C]-AA to labeled 6-keto-$PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2). Rings of aorta incubated in saline medium spontaneously formed PGI_2-like activity. This formation was dose-dependently inhibited by nicotine, with an I_{50} of about 10^{-4} M. It was also seen that platelet microsomes converted ^{14}C-AA to labeled TxB_2. This formation was unaffected by nicotine. In summary, it is concluded that a true difference in sensitivity to nicotine exists between cyclooxygenase in rabbit aorta and platelets. The data also demonstrate a tissue difference between rabbit aorta and platelets concerning their utilization of exogenous AA as substrate in the formation of platelet active compounds.

Alster, P. and *Wennmalm, Å.*

British Journal of Pharmacology 81:55-60, 1984.

Other support: Swedish Tobacco Company and Stiftelsen Clas Groschinskys Minnesfond.

From the Department of Clinical Physiology, Huddinge University Hospital, Huddinge, Sweden.

VI. *Immunology and Adaptive Mechanisms*

SUPPRESSOR T CELL ACTIVATION BY HUMAN LEUKOCYTE INTERFERON

The interferons are a group of proteins that inhibit viral replication and modulate a variety of immune responses. Murine fibroblast interferon (IFNβ) activates murine suppressor T lymphocytes *in vitro*, which suppress plaque-forming cell responses by spleen cells. Suppression of human *in vitro* immune responses by IFN was investigated here to determine whether human IFN also activates suppressor T cells. Human leukocyte IFN (IFNα) suppressed pokeweed mitogen-induced polyclonal immunoglobulin production by human peripheral blood mononuclear cells (PBMC) by 80–90% at doses of 200 to 350 U/ml. Responses by IFNα-treated PBMC were suppressed in a dose-dependent manner; control cultures had maximal responses on day 7. In other studies, PBMC incubated with 10,000 U/ml of IFNα contained activated suppressor cells that decreased pokeweed mitogen-stimulated polyclonal immunoglobulin production by autologous cells by 70–80%. Suppression mediated by these cells was prevented by catalase, ascorbic acid and 2-mercaptoethanol. Results from these and

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related investigations indicate that IFNα activates suppressor T cells in human PBMC cultures, while the ability of catalase, 2-mercaptoethanol, and ascorbic acid to block suppression suggests that these suppressor T cells have certain similarities to IFNβ or α concanavalin A-activated murine suppressor T cells.

Schnaper, H. W., *Aune, T. M.* and Pierce, C. W.

The Journal of Immunology 131(5):2301-2306, 1983.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Departments of Pathology and Microbiology-Immunology, and Pediatrics, Washington University School of Medicine, St. Louis.

CHARACTERIZATION AND MECHANISM OF ACTION OF SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS)

Soluble immune response suppressor (SIRS), a product of concanavalin A-activated murine Lyt 2^+ T cells, which nonspecifically suppresses immune responses *in vitro*, is also produced by Lyt 2^+ T cells activated with fibroblast interferon (IFNβ). The SIRS suppressor pathway, as currently perceived, is summarized in this paper. To note, Lyt 2^+ T cells, activated by con A or IFNβ, release SIRS. The target of SIRS is the macrophage which activates or oxidizes SIRS through a peroxide-mediated reaction. Catalase blocks conversion of SIRS to $SIRS_m$ by consuming peroxide. Levamisole also prevents conversion of SIRS to $SIRS_m$ by blocking activation of SIRS by peroxide. The mechanism of $SIRS_m$-mediated inhibition of cell division appears to involve oxidation of cellular protein sulfhydryl groups which is time-dependent, proportional to the amount of $SIRS_m$ added and prevented by dithiothreitol or 2-mercaptoethanol. The applicability of this pathway to immunosuppression or to inhibition of cell division in general remains to be determined. The finding that IFNβ activates this pathway and that levamisole blocks suppression by the SIRS pathway suggests that it may be an important host mechanism for regulating both immune responses and cellular proliferation in general.

Aune, T. M. and Pierce, C. W.

In: Hadden, J. W. *et al.* (eds.): *Advances in Immunopharmacology 2*, New York: Pergamon Press, 1983, pp. 597-602.

Other support: National Institute of Allergy and Infectious Diseases and the National Science Foundation.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and of Microbiology-Immunology, Washington University School of Medicine, St. Louis.

PROPERTIES OF THE SIRS SUPPRESSOR PATHWAY

In this study of the properties of the SIRS suppressor pathway, it was found that the SIRS suppressor pathway is initiated by activation of Ly 2^+ T lymphocytes by either con A or IFNβ. SIRS is a protein which has been purified and exists as two species with mol. wts. of 14,000 and 21,500. The target of SIRS is the macrophage and macrophages appear to oxidize or activate SIRS in a peroxide dependent process. Catalase blocks SIRS or IFNβ action by consuming H_2O_2 and levamisole blocks SIRS

or IFNB by preventing activati
block SIRS or IFNβ action in
$SIRS_m$ is a potent inhibitor of
neoplastic cells. The mechanism
to involve oxidation or modifica
levamisole have been found to a
ment of both of these substance
be an important host mechanis
proliferation in general.

Aune, T. H. and Pierce, C. W.

In: *13th International Cancer C*
Alan R. Liss, Inc., 1983, pp. 3

Other support: National Scien

From the Department of Pathol
St. Louis, and the Department o
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IDENTIFICATION AND INIT
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Schnaper, H. W., Pierce, C. W

The Journal of Immunology 132

Other support: Monsanto Com

From the Department of Pathol
St. Louis, and the Departments
School of Medicine, St. Louis.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

or IFNB by preventing activation or oxidation of SIRS by H_2O_2. Other agents which block SIRS or IFNβ action include electron donors which can inactivate $SIRS_m$. $SIRS_m$ is a potent inhibitor of immune responses and proliferation of normal and neoplastic cells. The mechanism of $SIRS_m$-mediated inhibition of proliferation appears to involve oxidation or modification of protein sulfhydryls. As of now, both IFNβ and levamisole have been found to affect a wide variety of cellular processes. The involvement of both of these substances in the SIRS pathway suggests that this pathway may be an important host mechanism for regulating both immune responses and cellular proliferation in general.

Aune, T. H. and Pierce, C. W.

In: *13th International Cancer Congress, Part B: Biology of Cancer (1)*, New York: Alan R. Liss, Inc., 1983, pp. 335-344.

Other support: National Science Foundation and the National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and of Microbiology-Immunology, Washington University School of Medicine, St. Louis.

IDENTIFICATION AND INITIAL CHARACTERIZATION OF CONCAVALIN A- AND INTERFERON-INDUCED HUMAN SUPPRESSOR FACTORS: EVIDENCE FOR A HUMAN EQUIVALENT OF MURINE SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS)

Antigen-nonspecific suppression in both murine and human systems may be mediated through several divergent pathways, through an ultimate common pathway, or through a combination of these two possibilities. In the paper presented here, it is noted that human suppressor T cells activated by leukocyte interferon have properties similar to murine suppressor cells activated by interferon or by concanavalin A. Murine suppressor cells release a soluble mediator, soluble immune response suppressor (SIRS), which accounts, at least in part, for suppressive activity in murine systems. To compare and contrast murine and human suppressor pathways, these investigators evaluated the suppression of human polyclonal plaque-forming cell responses by concanavalin A, by leukocyte interferon, and by immune interferon, or by suppressor cells activated by these agents. In each instance, suppressor cells released suppressor factors with similar properties that were indistinguishable from murine SIRS. As with murine SIRS, suppression by each of these human factors was inhibited by 2-mercaptoethanol, ascorbic acid, catalase, or levamisole. Significantly, the similarities between these human suppressor factors and murine SIRS show the existence of a human SIRS pathway.

Schnaper, H. W., Pierce, C. W. and *Aune, T. M.*

The Journal of Immunology 132(5):2429-2435, 1984.

Other support: Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Departments of Pathology and of Pediatrics, Washington University School of Medicine, St. Louis.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

MECHANISM OF SIRS ACTION AT THE CELLULAR AND BIOCHEMICAL LEVEL

In this comprehensive paper on SIRS, sections are devoted to T Cell Hybridomas Producing SIRS; Purification of SIRS; Mechanisms of Action of SIRS as regards: Inhibition of Immune Function; Inhibition of Cell Division; $SIRS_{ox}$-Mediated Cellular Protein Sulfhydryl Group Loss, and $SIRS_{ox}$-Mediated Inhibition of Microtubule Function. The discussion section of this paper points out that the SIRS suppressor pathway is initiated by activation of murine T lymphocytes with either concanavalin A or interferon β. Ly 2+ T lymphocytes release SIRS whose target is the macrophage. Macrophages appear to oxidize SIRS to $SIRS_{ox}$ in an H_2O_2-dependent process and $SIRS_{ox}$ is directly reponsible for inhibition of *in vitro* immune responses. Catalase prevents SIRS or IFNβ-mediated inhibition by competing with SIRS for H_2O_2. Similarly, the immunoenhancing drug, levamisole, inhibits IFNβ or SIRS-mediated suppression by inhibiting activation of SIRS by either macrophages or H_2O_2. Other observations considered in this discussion include: (1) SIRS/$SIRS_{ox}$ appears to be a general inhibitor of cellular proliferation. (2) $SIRS_{ox}$ appears to inhibit cell division by modifying certain protein sulfhydryl groups. (3) Certain features of $SIRS_{ox}$-catalyzed oxidation of protein sulfhydryl groups are similar to the enzymatic properties of sulfhydryl oxidase isolated from milk. (4) At this point it is not certain whether or to what extent the SIRS pathway participates in host regulation of immune responsiveness or cell division.

Aune, T. M. and Pierce, C. W.

In: *Lymphokines, Vol. 9*, New York: Academic Press, 1984, pp. 257-277.

Other support: National Science Foundation and the National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and Microbiology-Immunology, Washington University School of Medicine, St. Louis.

CATHEPSIN G IN HUMAN MONONUCLEAR PHAGOCYTES: COMPARISONS BETWEEN MONOCYTES AND U937 MONOCYTE-LIKE CELLS

Data demonstrating that U937 cells contain cathepsin G-like activity are presented in this report. U937 cells contain approximately 10μg of cathepsin G-like activity per 10^7 cells, about 25% of the cathepsin G activity in human neutrophils. Normal monocytes have minimal cathepsin G-like activity (approximately 0.1μg per 10^7 cells). The cathepsin G-like activity of U937 cells appears to be due to an enzyme that is the same as cathepsin G by several criteria: (1) it is a serine proteinase with activity like cathepsin G against a synthetic chymotrypsin substrate, succinyl-ala-ala-pro-phe-p-nitroanilide; (2) the proteolytic fragments it releases from fibronectin match those released by cathepsin G; (3) like cathepsin G, it can be purified by sequential Trasylol-Sepharose affinity chromatography and carboxymethyl-Sephadex ion exchange chromatography; (4) its amino acid composition and migration on SDS-polyacrylamide gel electrophoresis are indistinguishable from cathepsin G; and (5) it binds with antiserum raised to cathepsin G. The presence of cathepsin G in U937 cells, in much higher concentration than in normal monocytes, indicates either that the content of cathepsin G in monocytes decreases markedly during monocyte differentiation or

that U937 cells differ from n
this neutral proteinase.

Senior, R. M. and *Campbell,*

The Journal of Immunology 1

Other support: U. S. Public

From the Respiratory and Cr
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ANTIGENIC ANALYSIS O
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Strauss, L. C., Stuart, R. K.

Blood 61(6):1222-1231, 1983.

Other support: National Inst

From the Department of Onc
Hematology, Johns Hopkins

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that U937 cells differ from normal immature monocytes with respect to synthesis of this neutral proteinase.

Senior, R. M. and *Campbell, E. J.*

The Journal of Immunology 132(5):2547-2551, 1984.

Other support: U. S. Public Health Service.

From the Respiratory and Critical Care Division, Department of Medicine, Jewish Hospital at Washington University Medical Center, St. Louis.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS. I. EXPRESSION OF THE MY-1 GRANULOCYTE SURFACE ANTIGEN ON HUMAN MARROW CELLS AND LEUKEMIC CELL LINES

Plasma membrane components appear to mediate many granulocyte functions. For example, cell surface receptors for the Fc region of immunoglobulin and for the third component of complement play roles in granulocyte phagocytosis and antibody-mediated cellular cytotoxicity. These receptors are not granulocyte-specific, however, since they are expressed on cells of other lineages. The existence of human neutrophil-specific antigens has been inferred from studies of immune granulo-cytopenia. In the study reported here, five monoclonal antibodies that identify the My-1 human granulocyte surface antigen were not reactive with other peripheral blood cells. These antibodies affected complement-dependent cytolysis of a large fraction of normal human marrow leukocytes. This My-1-positive marrow cell population consisted of morphologically identifiable granulocytic precursor cells. Colony-forming cells of the granulocyte-monocyte lineage (CFC-GM) did not express My-1, suggesting that the My-1 antigen is expressed later in normal granulocytic maturation. However, these antibodies did react with myeloid leukemia cell lines. The significance and potential utility of these probes for the understanding of granulopoietic differentiation is discussed.

Strauss, L. C., Stuart, R. K., and *Civin, C. I.*

Blood 61(6):1222-1231, 1983.

Other support: National Institutes of Health and the Heart of Variety Fund.

From the Department of Oncology, Divisions of Pediatric Oncology and Experimental Hematology, Johns Hopkins University School of Medicine, Baltimore.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS: II. EXPRESSION OF HUMAN NEUTROPHIL ANTIGENS ON NORMAL AND LEUKEMIC MARROW CELLS

Hybridoma-derived monoclonal antibodies (McAb) specifically reactive with lymphocyte cell surface molecules have been of great value in the analysis of lymphocyte differentiation and lymphoid neoplasia. McAb reactive with human neutrophils have been developed, and antibodies against the My-1 human granulocyte antigen have been shown to react with morphologically identifiable neutrophil precursors, but not with colony-forming cells of the granulocyte-monocyte lineage (CFC-GM). In the

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work reported here, the binding of five antineutrophil monoclonal antibodies AHN-1, -2, -3, -7, and -8, to normal and leukemic bone marrow cells was studied. AHN-7 bound to many granulocytic precursors, particularly myelocytes, and both lymphoid and blast cells in normal marrow, and to most but not all granulocyte-macrophage progenitors (CFC-GM). AHN-8 bound only to late (band and segmented) neutrophilic cells and not to CFC-GM. AHN-1, -2, -3 bound to morphologically identifiable neutrophil precursors, but not to (day-14) CFC-GM. Approximately half of non-lymphoid leukemia specimens were positive with AHN-1 or AHN-7; by contrast, lymphoid leukemia specimens were rarely positive. AHN-8 was rarely found on leukemia cells. These antineutrophil antibodies appear to detect distinct granulopoietic subsets and may be useful in the analysis of hematologic differentiation and in the subclassification of leukemias.

Strauss, L. C., Skunitz, K. M., August, J. T., and *Civin, C. I.*

Blood 63(3):574-578, 1984.

Other support: National Institutes of Health, Heart of Variety Fund and the Johnson & Johnson Company.

From the Department of Oncology, Division of Pediatric Oncology, and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS. III. A HEMATOPOIETIC PROGENITOR CELL SURFACE ANTIGEN DEFINED BY A MONOCLONAL ANTIBODY RAISED AGAINST KG-1A CELLS.

In this study, the KG-1a human leukemic cell line was used as an immunogen in an attempt to produce antibodies against human blast cell-surface antigens. The KG-1 myeloblastic leukemic cell line was derived from a patient with nonlymphocytic leukemia and the KG-1a cell line arose from it as a spontaneous tissue culture variant. As reported here, the anti-My-10 mouse monoclonal antibody was raised against the immature human lyeloid cell line KG-1a and was selected for nonreactivity with mature human granulocytes. Anti-My-10 immunoprecipitated a KG-1a cell surface protein with an apparent Mr of approximately 115 kD. The authors describe the binding of this antibody to human hematopoietic cell types and show that My-10 is expressed specifically on immature normal human marrow cells, including hematopoietic progenitor cells. My-10 is also expressed by leukemic marrow cells from a subpopulation of patients. Thus, this antibody allows the identification and purification of hematopoietic progenitor cells from normal human marrow and the subclassification of leukemia.

Civin, C. I. et al.

The Journal of Immunology 133(1):157-165, 1984.

Other support: National Institutes of Health and the Heart of Variety Fund.

From the Johns Hopkins Oncology Center, Divisions of Pediatric Oncology and Cell Structure and Function; and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore.

RAPID, EFFICIENT CLON
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Other support: National Ins
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A SYNTHETIC PEPTIDE I

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Seiden, M. V., *Clevinger, B.*

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RAPID, EFFICIENT CLONING OF MURINE HYBRIDOMA CELLS IN LOW GELATION TEMPERATURE AGAROSE

This paper describes a simple method for cloning of hybridomas that is rapid, reliable and efficient in terms of cost, time and cloned hybridoma recovery. The method utilizes highly-purified, extremely low-gelation temperature agarose that can be used for extended periods of time at room temperature without gelation. To be specific, the use of ultra-low gelation temperature agarose allowed the investigators to avoid placing a water bath, a potential source of microbial contamination, in their laminar flow hood. More important, cells never needed to be exposed to potentially toxic temperatures, >37°C. In addition, several lots of this agarose have been tried and were not toxic to hybridoma cells. Cloning time and efforts were reduced by avoiding the need for constant manipulations to maintain medium temperature above 37°C to prevent gelation of conventional agarose. Five or more hybridomas can easily be cloned in an hour using this method. In this study, cloning efficiency varied from hybridoma to hybridoma. However, it was found that clones which were proliferating rapidly and were highly viable routinely had cloning efficiencies higher, by orders of magnitude, than clones which were growing poorly in liquid cultures.

Civin, C. I. and Banquerigo, M. L.

Journal of Immunological Methods 61:1-8, 1983.

Other support: National Institutes of Health, the Blood Systems, and the Heart of Variety Fund.

From the Department of Oncology, Division of Pediatric Oncology, Johns Hopkins University School of Medicine, Baltimore.

A SYNTHETIC PEPTIDE INDUCES A NEW ANTI-DEXTRAN IDIOTYPE

One of the major factors confounding attempts to make serologic and molecular correlations is the complexity of the idiotypic determinants defined by conventional serologic techniques. There has recently been a new approach to this problem, though, since peptides which mimic the primary sequences of numerous antigens have been used to generate antibodies which bind both peptide and the whole molecules from which the peptide was derived. In the summary of the paper presented here, it is shown that the J_H1 peptide has been synthesized and coupled to keyhole limpet hemocyanin for use as an immunogen. A serum from an animal hyperimmunized with this immunogen demonstrated binding to both peptide and native immunoglobulin. Furthermore, this serum seems to define an idiotypic determinant nearly unique to $\alpha(1\rightarrow3)$ dextran-binding immunoglobulins. Synthetic peptide immunogens may be a useful tool in probing the molecular basis of idiotypy.

Seiden, M. V., *Clevinger, B.*, Srouji, T., Davie, J. M., McMillan, S., and Lerner, R.

Annales d'Immunologie (Inst. Pasteur) 135:77-82, 1984.

Other support: National Institutes of Health.

From the Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis; Department of Biomedical Science, Washington University School of Dental Medicine, St. Louis, and Department of Molecular Biology, the Research Institute of Scripps Clinic, La Jolla, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

CHEMICAL SYNTHESIS OF IDIOTOPES: EVIDENCE THAT ANTISERA TO THE SAME JH_1 PEPTIDE DETECT MULTIPLE BINDING SITE-ASSOCIATED IDIOTOPES

More than 20 years ago, Kunkel, H. G., Mannik, M. and Williams, R. C. (1963) showed that the structural diversity expressed by variable region domains could be detected by antisera. These variable region antigenic determinants, called idiotopes, provide simple and highly specific ways of identifying and comparing variable regions and have been widely used in immunological research. In addition to providing useful markers for investigating variable region structure and function, idiotypes may provide an important means of immune regulation. To better understand the molecular basis of idiotypy, the investigators have generated several site-specific antisera through immunization of animals with synthetic peptides corresponding to the (JH_1) heavy chain joining segment 1 of the mouse heavy chain variable (V_H) region. These antipeptide sera identify several idiotypic determinants present on intact hybridoma and myeloma immunoglobulins. Expression of at least three of these idiotopes is correlated with the antigen specificity of the family of immunoglobulins bearing the determinant. Use of synthetic peptides may prove a powerful technique in the generation of molecularly defined antiidiotypic reagents.

Seiden, M. V., *Clevinger, B.*, McMillan, S., Srouji, A., Lerner, R., and Davie, J. M.

Journal of Experimental Medicine 159:1338-1350, 1984.

Other support: U. S. Public Health Service.

From the Department of Microbiology and Immunology, School of Medicine, and Division of Biomedical Science, School of Dental Medicine, Washington University, St. Louis, and the Department of Molecular Biology, Research Institute of the Scripps Clinic, La Jolla, CA.

RADIOENZYMATIC ASSAY FOR MEASUREMENT OF TISSUE CONCENTRATIONS OF HISTAMINE: ADAPTATION TO CORRECT FOR ADHERENCE OF HISTAMINE TO MECHANICAL HOMOGENIZERS

Measurements of histamine concentrations in tissue have important experimental applications. Because adherence of histamine to glass is well-known, the present investigators tested for its adherence to a mechanical homogenizer commonly used in the extraction of histamine from tissue samples. During 60 sec of homogenization, 15% to 71% of the histamine originally present in the samples "disappeared" and the reason for the disappearance was reversible binding of histamine to the homogenizer. Adding trace amounts of [^{3}C] histamine to each sample before homogenization and measuring the disappearance of radioactivity during homogenization permitted correction for binding to the homogenizer. This technique for correction was validated by the measurement of endogenous concentrations of histamine in the tracheal posterior membranes of six dogs (range of mean concentrations: 0.63 to 1.51 ng/mg wet weight) followed by the measurement of known amounts of exogenous histamine added before homogenization to tracheal tissue samples from the same dogs. In the latter samples, 96 ± 13% (mean ± SEM) of the histamine added was measured by this technique. The authors conclude that binding of histamine to mechanical homogenizers may be an

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Brown, J. K., Frey, M. J., Reed

The Journal of Allergy and Clini

Other support: U. S. Public He

From the Cardiovascular Researc
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IMBALANCES IN SUBSETS O
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Other support: National Institu
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From the Departments of Pedi
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Health Science Center, Dallas.

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important cause of inaccuracy of the enzymatic assay for the measurement of histamine concentrations in tissue but that such binding may be easily corrected for.

Brown, J. K., Frey, M. J., Reed, B. R., Leff, A. R., Shields, R., and *Gold, W. M.*

The Journal of Allergy and Clinical Immunology 73(4):473-478, 1984.

Other support: U. S. Public Health Service.

From the Cardiovascular Research Institute and the Department of Medicine, University of California, San Francisco, and Respiratory Care Section, Department of Medicine, Veterans Administration Medical Center, San Francisco.

IMBALANCES IN SUBSETS OF T LYMPHOCYTES IN AN INBRED PEDIGREE WITH OMENN'S SYNDROME

This report describes the immunologic function and immunoregulatory subsets of T lymphocytes in two affected infants and 109 healthy family members of the same pedigree originally described by Omenn. Eighteen homozygotes in this pedigree had previously died from infection at less than six months of age. Both of the infants described here displayed normal numbers of peripheral blood T (E-rosette) lymphocytes, poor mitogen reactivity of lymphocytes, normal mixed lymphocyte culture reactivity, a paucity of circulating B cells, variable hypogammaglobulinemia, and elevated serum IgE concentrations. At four months of age, one infant (boy) had 95% T3+ (total T) peripheral blood lymphocytes, 41% T4+ (helper T) lymphocytes, and 64% T8+ (suppressor T) lymphocytes; at four months of age the other infant (girl) had 43% T3+, 43% T4+, 19% T8+ lymphocytes, and 18% T6+ (stage II thymocyte) lymphocytes. Age-matched controls had values 49% for T3+, 37% for T4+, 13% for T8+, and <1% T6+. Functional lymphocyte suppression assayed by the reverse hemolytic plaque assay indicated that the infant girl's lymphocytes suppressed 75% of the immunoglobulin produced by normal lymphocytes. The 109 healthy family members (containing many obligate heterozygotes) were analyzed for distributions of T-lymphocyte subsets and compared with 37 age-matched controls. Based on these results, it was concluded that the immunodeficiency in Omenn's syndrome is due to deregulation of T-lymphocyte subsets, appearance of immature T cells in the peripheral blood, functional T-cell suppression of immunoglobulin production, and reduced B-cell populations. An abnormal distribution of the percentage of T4- and T8-positive cells, which exists in an extraordinary number of family members, may serve as a phenotypic lymphocyte marker and thereby aid in the identification of the heterozygous state.

Karol, R. A., Eng, J., Cooper, J. B., Dennison, D. K., Sawyer, M. K., *Lawrence, E. C.*, Marcus, D. M., and Shearer, W. T.

Clinical Immunology and Immunopathology 27:412-427, 1983.

Other support: National Institutes of Health, General Clinical Research Center, John P. McGovern Foundation, and the Texas Children's Hospital.

From the Departments of Pediatrics, Medicine, and Microbiology and Immunology, Baylor College of Medicine, and the Allergy and Immunology Service, Texas Children's Hospital, Houston, and the Department of Pediatrics, University of Texas Health Science Center, Dallas.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

INEFFECTIVE IMMUNOGLOBULIN SECRETION IN RESPONSE TO POKEWEED MITOGEN IN SARCOIDOSIS

Sarcoidosis is a systemic granulomatous disease of unknown cause associated with alterations in both cellular and humoral immune functions. Recently, monocytes which suppress pokeweed mitogen (PWM)-induced specific antibody formation have been reported in sarcoidosis. The purpose of the current study was to evaluate whether suppressor monocytes might also be operative for polyclonal immunoglobulin (Ig) secretion in this disease. Accordingly, peripheral blood mononuclear cells (MNL) from sarcoid patients and normal control subjects were cultured with PWM for six days and the total number of Ig-secreting cells (Ig-SC) determined with a reverse hemolytic plaque assay. While normal MNL responded to PWM with a 10-fold or greater increase in Ig-SC, sarcoid MNL failed to respond to PWM. Sarcoid MNL contained greater percentages of monocytes than normals (44.8 ± 2.0% vs. 30.4 ± 1.4%, $p < 0.001$) and there was a negative correlation between the magnitude of the response to PWM and the percentage of MNL-monocytes. However, prior removal of monocytes improved the responsiveness to PWM in only four patients. Excessive suppressor monocyte activity in co-culture studies could be identified in only these same four patients. Thus, neither a relative monocytosis, while present in sarcoidosis, nor excessive suppressor monocyte activity may entirely account for the hyporesponsiveness to PWM.

Lawrence, E. C. et al.

In: Chretien, J., Marsac, J., and Saltiel, J. C. (eds.): *Sarcoidosis*, New York: Pergamon Press, 1981, pp. 98-102.

Other support: Gulf Oil Foundation and the American Lung Association.

From The Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of the Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

CORRELATION OF DISEASE ACTIVITY IN SARCOIDOSIS WITH SERUM ANGIOTENSIN CONVERTING ENZYME, ^{67}GALLIUM LUNG SCANNING AND BRONCHOALVEOLAR LAVAGE

As stated in this paper, sarcoidosis is a granulomatous disease of unknown cause which is often treated with corticosteroids to relieve symptoms and to suppress inflammatory lung involvement. Gallium-67 (^{67}Ga) lung scanning, serum angiotensin-converting enzyme (SACE), and bronchoalveolar lavage (BAL) have been proposed as useful techniques to be followed in the assessment of disease activity and response to therapy in sarcoidosis. The purpose of the present study was to compare these various procedures with clinical assessments of disease activity in 87 studies performed over an 18-month period on 26 patients with sarcoidosis. While there were strong statistical associations between BAL, ^{67}Ga lung scanning, SACE levels and clinical assessments of disease activity, each parameter was useful in different ways. Thus, greater than 20% BAL-lymphocytes was associated with disease activity, whereas less than 3000 BAL-IgG secreting cells/10^6 BAL lymphocytes was always indicative of inactive disease. By contrast, SACE levels of 750 U/ml were always associated with disease activity. Quantitation of ^{67}Ga lung scanning correlated very well with clinical assessments of disease activity in both treated and untreated patients, with only rare false positive or false negative results. No single parameter may be infallible in the assess-

ment of disease activity in sarco
scanning and BAL analysis may

Lawrence, E. C. et al.

In: Cheretien, J., Marsac, J., ar

Pergamon Press, 1981, pp. 430-

Other support: Gulf Oil Found

From Rockwell-Keough Pulmor
Research Center of the Method
College of Medicine, Houston.

GANGLIOSIDES OF HUMA

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Westrick, M. A., Lee, W. M

Biochimica et Biophysica Act

Other support: Cancer Resea

From the Cancer Research
Pharmaceutical Chemistry, U

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

ment of disease activity in sarcoidosis but determination of SACE levels, ^{67}Ga lung scanning and BAL analysis may be complementary toward this goal.

Lawrence, E. C. et al.

In: Cheretien, J., Marsac, J., and Saltiel, J. C. (eds.): *Sarcoidosis*, New York: Pergamon Press, 1981, pp. 430-433.

Other support: Gulf Oil Foundation and the American Lung Association.

From Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of the Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

GANGLIOSIDES OF HUMAN ACUTE LEUKEMIA CELLS

In an attempt to gain more information on the gangliosides present in acute leukemia cells, these investigators characterized the gangliosides from cells of eight patients with different forms of acute leukemia (four lymphoblastic, four nonlymphoblastic) by thin-layer chromatography and high-performance liquid chromatography combined with glycosidase treatment. Their analysis indicated both quantitative and qualitative differences between the gangliosides of acute leukemia and those of normal leukocytes: (1) the absolute amount of ganglioside was decreased in the acute leukemia cells; (2) in general, acute leukemias had a more simplified ganglioside pattern in that they contained a greater proportion of the short-chain ganglioside, II^{3}NeuAc-LacCer (GM3); (3) all of the acute leukemia cells contained reduced quantities of the ganglioside *N*-acetylneuraminosyllactotriaosylceramide, a compound previously found only in normal leukocytes, and (4) a disialylated ganglioside, II^3(NeuAc)$_2$-LacCer (GD3), which is not found in normal leukocytes, was isolated from the cells of one patient with acute nonlymphoblastic leukemia. These findings demonstrate important differences between the gangliosides of acute leukemia cells and normal leukocytes.

Westrick, M. A., Lee, W. M. F., Goff, B., and *Macher, B. A.*

Biochimica et Biophysica Acta 750:141-148, 1983.

Other support: Cancer Research Funds of the University of California.

From the Cancer Research Institute, Department of Medicine and Department of Pharmaceutical Chemistry, University of California, San Francisco.

GANGLIOSIDES OF HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA AND HAIRY CELLS

Gangliosides are an important subclass of glycosphingolipids which contain sialic acid. The research efforts of this laboratory have been focused on the quantification and structural characterization of neutral glycosphingolipids and gangliosides from human leukocytes and leukemia cells. In the study reported here, gangliosides were purified from the cells of two patients with hairy cell leukemia and one patient with

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

chronic lymphocytic leukemia. Quantification of these compounds showed that these cells contain only 5-15% of the amount of lipid-bound sialic acid (gangliosides) per cell as normal lymphocytes. Structural characterization by gas-liquid chromatography, glycosidase treatment and high-performance liquid chromatography demonstrated that the major gangliosides of these leukemia cells were of the lactosyl type. Hairy cells contained monosialyl-lactosylceramide (II^{3}NeuAc-LacCer); whereas chronic lymphocytic leukemia cells contained both monosialyl and disialyl lactosylceramide [II^3(NeuAc)$_2$-LacCer]. Chronic lymphocytic leukemia cells contained lesser amounts of three other gangliosides of the neolacto or lacto series as determined by endo-β-galactosidase treatment. None of these leukemia cells contained detectable quantities of NeuAc-LcOse$_4$Cer, a ganglioside found in normal leukocytes.

Goff, B. A., Lee, W. M. F., Westrick, M. A., and *Macher, B. A.*

European Journal of Biochemistry 130:553-557, 1983.

Other support: National Institutes of Health and the National Cancer Institute.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

ISOLATION AND CHARACTERIZATION OF GANGLIOSIDES FROM CHRONIC MYELOGENOUS LEUKEMIA CELLS

The purpose of this study was to isolate and structurally characterize the major chronic myelogenous leukemia (CML) gangliosides and compare them to the gangliosides of normal neutrophils. As presented here, gangliosides isolated from the cells of three patients with CML were purified by Folch partitioning, diethylaminoethyl Sephadex, Florisil (acetylated gangliosides), and silicic acid chromatography and were structurally analyzed using thin-layer and gas-liquid chromatography, methylation analysis, enzyme degradation, and high-performance liquid chromatography. With these methods, the major gangliosides isolated were II^3-α-N-acetylneuraminosyllactosylceramide, IV^3-α-acetylneuraminosyl-neolactotetraosylceramide (sialosylparagloboside), and a ganglioside with the following structure: NeuAcα2→3(Galβ1→4GlcNacβ1→3)$_2$Galβ1→4Glcβ1→1Cer. This ganglioside has previously been characterized as an "i" active compound. It was also found that, like normal neutrophils, CML cells contain monosialogangliasides that belong to the lactosyl and neolacto family. However, this study shows that CML cells differed from normal neutrophils in that they contained less total ganglioside, and their major ganglioside species is II^3-α-N-acetylneuraminosyllactosylceramide. Difference between gangliosides of CML and acute nonlymphoblastic leukemias are discussed.

Westrick, M. A., Lee, W. M. F., and *Macher, B. A.*

Cancer Research 43:5890-5894, 1983.

Other support: National Cancer Institute.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

ASSOCIATION OF GANGLIOSIDE AND SENDAI VIRUS: REQUIRE VIRAL FUSION

In this study, a method is descri in particular antibodies and their fr The protein-ganglioside conjugate i separated from free protein by mole versibly transfer from the micelle to be identified as a new surface antige has been demonstrated with three b gate has been transferred to humar glutinated with goat anti-rabbit Ig H2K^k have been shown to adhere express H2K^k-antigen. Mouse mon associate with Sendai virus and co hemolyze desialylated human ery these investigators demonstrated th for viral binding, appears also to be eliminates hemolysis and fusion, virus.

Heath, T. D., Martin, F. J. and *M*

Experimental Cell Research 149:16

Other support: National Cancer In

From the Cancer Research Institu University of California, San Fran

DIFFERENTIAL EXPRESSION LEUKOCYTES AND LEUKEMI

Earlier analyses of the glycos mia cells have shown that these c but only one disialoganglioside, (leukocytes and the cells of 25 pati the presence of disialoganglio neuraminosyllactosylceramide (G chromatographs with an anti-G_{D3} cells tested, acute leukemia cells v and normal neutrophils did not apparent within the acute myelo leukemia cells stained more inten characteristics. All lymphocytic l this ganglioside could not be ganglioside extract from the cells for G_{D3} immunostaining. These re myelogenous leukemia cells are leukemia cells on the basis of G_{D}

Siddiqui, B., Buehler, J., DeGr

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

ASSOCIATION OF GANGLIOSIDE-PROTEIN CONJUGATES INTO CELL AND SENDAI VIRUS: REQUIREMENT FOR THE HN SUBUNIT IN VIRAL FUSION

In this study, a method is described for preparing a covalent conjugate of proteins, in particular antibodies and their fragments, with gangliosides in the micellar form. The protein-ganglioside conjugate is associated with ganglioside micelles and can be separated from free protein by molecular sieve chromatography. Conjugates can irreversibly transfer from the micelle to a cell membrane of choice, and the protein portion be identified as a new surface antigen. The successful application of this methodology has been demonstrated with three biological systems. Rabbit IgG-ganglioside conjugate has been transferred to human or sheep erythrocytes, which have been hemagglutinated with goat anti-rabbit IgG. Erythrocytes modified with ganglioside-anti-H2K^{k} have been shown to adhere to monolayers of L929 mouse fibroblasts which express H2K^{k}-antigen. Mouse monoclonal antiglycophorin ganglioside conjugate can associate with Sendai virus and confer upon the virus the ability to agglutinate and hemolyze desialylated human erythrocytes. Using the antiglycophorin conjugate, these investigators demonstrated that the HN subunit, which is normally responsible for viral binding, appears also to be essential for fusion activity because its destruction eliminates hemolysis and fusion, but not agglutination, by the conjugate-modified virus.

Heath, T. D., Martin, F. J. and *Macher, B. A.*

Experimental Cell Research 149:163-175, 1983.

Other support: National Cancer Institute and the National Institutes of Health.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

DIFFERENTIAL EXPRESSION OF GANGLIOSIDE G_{D3} BY HUMAN LEUKOCYTES AND LEUKEMIA CELLS

Earlier analyses of the glycosphingolipids of normal human leukocytes and leukemia cells have shown that these cells contain several types of monosialogangliosides but only one disialoganglioside, G_{D3}. In the present study, gangliosides from normal leukocytes and the cells of 25 patients with acute and chronic leukemia were tested for the presence of disialoganglioside II^3-α-*N*-acetylneuraminosyl-α2→8-*N*-acetylneuraminosyllactosylceramide (G_{D3}). G_{D3} was detected by immunostaining thin-layer chromatographs with an anti-G_{D3} monoclonal antibody (AbR_{24}). Among the myeloid cells tested, acute leukemia cells were positive for G_{D3}, whereas chronic leukemia cells and normal neutrophils did not have detectable G_{D3}. A range of G_{D3} reactivity was apparent within the acute myeloid leukemia cells; gangliosides from pure myeloid leukemia cells stained more intensely than those from leukemia cells with monocytic characteristics. All lymphocytic leukemia cells (chronic and acute) contained G_{D3}, but this ganglioside could not be detected in extracts from normal lymphocytes. A ganglioside extract from the cells of a patient with hairy cell leukemia was also positive for G_{D3} immunostaining. These results demonstrate that normal leukocytes and chronic myelogenous leukemia cells are distinguished from other lymphoid and nonlymphoid leukemia cells on the basis of G_{D3} ganglioside expression.

Siddiqui, B., Buehler, J., DeGregorio, M. W., and *Macher, B. A.*

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Cancer Research 44:5262-5265, 1984.

Other support: National Cancer Institute and Louis R. Lurie Foundation.

From the Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco; Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco; and Children's Cancer Research Institute, Pacific Medical Center, San Francisco.

INHIBITION OF MITOGEN- AND ANTIGEN-INDUCED LYMPHOCYTE ACTIVATION BY HUMAN LEUKEMIA CELL GANGLIOSIDES

This study demonstrated that gangliosides prepared from human leukemia cells inhibit mitogen and antigen activation of human lymphocytes. Furthermore, the investigators have analyzed the effect of purified and structurally defined gangliosides on lymphocyte blastogenesis. These analyses allow them to conclude that: (a) gangliosides of human leukemia cells, when added to *in vitro* assay systems in concentrations known to occur in the serum of humans with cancers, suppress lymphocyte activation; (b) each purified ganglioside suppressed blastogenesis to a similar extent; and (c) sialic acid is essential for maximal immunosuppression. The three gangliosides used in this study are also known to be components of normal cells and therefore should not be considered leukemia-associated components. However, the concentration of these compounds is known to be elevated in the serum of patients with cancers and, therefore, may contribute to a reduced immune response in some cancer patients.

Gonwa, T. A., Westrick, M. A., and *Macher, B. A.*

Cancer Research 44:3467-3470, 1984.

Other support: National Cancer Institute, U. S. Public Health Service, and National Research Service Awards.

From the Department of Medicine, University of Iowa, Iowa City, and Cancer Research Institute and the Department of Pharmaceutical Chemistry, University of California, San Francisco.

EXPRESSION OF HLA-DR BY A HUMAN MONOCYTE CELL LINE IS UNDER TRANSCRIPTIONAL CONTROL

In this attempt to determine the mechanism by which Ia induction occurs, experiments were designed to investigate the molecular events leading to expression of the human Ia molecule, HLA-DR. To accomplish this, the human monocytoid cell line U 937, which does not express any detectable HLA-DR molecules, was used. Utilizing a cDNA probe for the α chain of HLA-DR and total cellular RNA, it could be demonstrated that resting U 937 lacked detectable HLA-DR transcripts. Digestion of genomic DNA from U 937 with the isoschizomers Msp I and Hpa II followed by analysis of the restriction fragments on Southern blots demonstrated the HLA-DR α chain genes to be methylated. Addition of 5-azacytidine, an analogue of cytidine which causes hypomethylation of DNA to U 937 caused hypomethylation of HLA-DR α chain genes but did not, by itself, lead to the appearance of HLA-DR molecules on transcripts. However, treatment of U 937 with 5-azacytidine followed by addition of either culture

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The results of these studies provic
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Peterlin, B. M., Gonwa, T. A. a

Journal of Molecular Cell Immu

Other support: U. S. Public He

From the Howard Hughes Medic
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BOMBESIN AND VASOACTI DEVELOPING LUNG: MARK DISTRESS SYNDROME

Because there have been no
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concentration remained almost u
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contrast to bombesin, VIP was
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Ghatei, M. A., Sheppard, M. N
and Bloom, S. R.

Journal of Clinical Endocrinol

Other support: The Medical Re

From the Departments of Medic
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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

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fluids from activated T cells or human recombinant γ interferon did lead to the rapid appearance of abundant, mature HLA-DR transcripts and surface HLA-DR molecules. The results of these studies provide the first demonstration that methylation plays a role in the expression of human Ir genes and that induced expression of Ia molecules by soluble factors from T cells, including γ interferon, is accompanied by the rapid appearance of Ir gene transcripts.

Peterlin, B. M., Gonwa, T. A. and Stobo, J. D.

Journal of Molecular Cell Immunology 1:191-200; 1984.

Other support: U. S. Public Health Service.

From the Howard Hughes Medical Institute and the Department of Medicine, University of California, San Francisco.

BOMBESIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IN THE DEVELOPING LUNG: MARKED CHANGES IN ACUTE RESPIRATORY DISTRESS SYNDROME

Because there have been no immunological studies on the fetal or neonatal development of vasoactive intestinal polypeptide (VIP) in human lung or in lung diseases, in the present study the quantitative distribution of bombesin- and VIP-like immunoreactivities was determined by RIA and immunocytochemistry in regions of trachea, bronchus, and whole lung at various stages of human fetal development and in neonates, children, and adults. In addition, these two immunoreactivities were studied in infants that had died of the acute respiratory distress syndrome. Results showed that the concentration of bombesin-like immunoreactivity in the whole respiratory tract steadily increased during gestation, reaching a plateau at birth. In the lung, bombesin concentration remained almost unchanged during childhood but decreased to one tenth in the adult. In neonates with the acute respiratory distress syndrome, there was a significantly lower bombesin content in all regions of the respiratory tract compared to either normal full-term infants or 24- to 28-week-old fetuses. In related studies, immunocytochemistry localized bombesin immunoreactivity within mucosal neuroendocrine cells present in the airway epithelium throughout the respiratory tract and particularly in the intrapulmonary airways. The number of cells increased throughout gestation, reflecting the pattern found by RIA, and was greatly decreased in acute respiratory distress syndrome patients. Also, VIP concentrations were much lower than those of bombesin and did not change significantly with gestational age. In contrast to bombesin, VIP was mainly concentrated in the upper respiratory tract. In infants with the respiratory distress syndrome, the VIP content was not different from normal. These results are compatible with the possibility that bombesin-like peptides may have a role in the normal development of the human lung.

Ghatei, M. A., Sheppard, M. N., Henzen-Logman, S., Blank, M. A., *Polak, J. M.*, and Bloom, S. R.

Journal of Clinical Endocrinology and Metabolism 57(6):1226-1232; 1983.

Other support: The Medical Research Council and the Wellcome Trust.

From the Departments of Medicine and Histochemistry, Hammersmith Hospital, London, England

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

REGULATORY PEPTIDES: LOCALIZATION AND MEASUREMENT

The presence of five regulatory peptides, vasoactive intestinal peptide (VIP), substance P, bombesin (BN), cholecystokinin (CCK), and somatostatin, was investigated within various tissue structures, using immunocytochemistry for their localization and radioimmunoassay for the precise measurement and chemical characterization. Studies were conducted in neonatal and adult humans, rats, guinea pigs, and cats. Histologic demonstration of the diffuse neuroendocrine system in its entirety was carried out using antibodies to neuron specific enolase (NSE); delineation of the two components, neural and glial, of the autonomic nervous system was accomplished by the combined use of antisera to two brain proteins: NSE (for autonomic nerves) and S100 (for glial cells). VIP and substance P were the most abundant peptides and were localized to autonomic nerves, mainly in the upper respiratory tract, including the nasal mucosa. VIP was frequently found to be associated with secretory glands, smooth muscle and blood vessels, whereas substance P was often seen in close association with bronchial epithelium. VIP nerve fibers were seen to have a dual origin, from local cell bodies found almost exclusively in the wall of the trachea and from the sphenopalatine ganglion, where numerous VIP-containing neurons could be detected. Also, production of regulatory peptides, principally bombesin, was noted in lung endocrine tumors (i.e., small cell carcinoma) characterized immunohistologically by their high content of NSE and ultrastructurally by the presence of recognizable secretory granules. The exciting discovery of a large number of regulatory peptides in most peripheral tissues has promoted the lung to its well-deserved rank as one of the organs best provided with the diffuse neuroendocrine system. Investigations of the precise distribution and tissue localization of regulatory peptides throughout the respiratory tract and within each tissue structure provide full support of the increasingly accepted view of pulmonary regulation by messenger substances.

Polak, J. M. and Bloom, S. R.

In: Becker, K. L. and Gazdar, A. F. (eds.): *The Endocrine Lung in Health and Disease*, Philadelphia, W. B. Saunders Company, 1984, pp. 300-327.

From the Departments of Medicine and Histochemistry, Hammersmith Hospital, London, England.

BIOSYNTHESIS AND ASSEMBLY OF THE α AND β SUBUNITS OF Mac-1, A MACROPHAGE GLYCOPROTEIN ASSOCIATED WITH COMPLETE RECEPTOR FUNCTION

As reported previously, Mac-1 is a macrophage surface antigen containing noncovalently associated α and β subunits of M_r = 170,000 and 95,000, respectively. To determine whether the subunits are derived from a common or separate precursor, the biosynthesis of Mac-1 was studied. [^{35}S]Methionine pulse-chase-labeled material was immunoprecipitated with either a monoclonal antibody recognizing an α chain determinant present in the associated α,β, complex or a polyclonal antiserum recognizing the α,β, complex as well as the free β subunit. In peritoneal exudate macrophages, the α subunit was derived from a precursor of M_r = 161,000 which was converted to the mature M_r = 170,000 chain with a $t^{1/2}$ of 30 to 45 min. The β subunit was derived from a M_r = 87,000 precursor which became associated with the α subunit and was converted to M_r = 95,000 with a $t^{1/2}$ of 2 h. Labeled β chain took longer than α to become

associated with the α,β, con
phage populations, correlati
phage-like tumor line, α and
β precursors were present i
ceeded processing.

Ho, M-K. and *Springer, T.*

The Journal of Biological C

Other support: U. S. Public

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DENDRITIC CELL AND M
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Flotte, T. J., *Springer, T.*

American Journal of Patho

Other support: National In
Sciences and the American

From the Department of Pa
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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

SUREMENT

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BUNITS OF Mac-1, A
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associated with the α,β complex in a number of different types of peritoneal macrophage populations, correlating with synthesis of an excess of β. In the P388D$_1$ macrophage-like tumor line, α and β were processed with $t^1/_2$s of about 2 and 1h. Both α and β precursors were present in the complex, suggesting that complex formation preceeded processing.

Ho, M-K., and *Springer, T. A.*

The Journal of Biological Chemistry 258(5):2766-2769, 1983.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, and the Department of Pathology, Harvard Medical School, Boston.

DENDRITIC CELL AND MACROPHAGE STAINING BY MONOCLONAL ANTIBODIES IN TISSUE SECTIONS AND EPIDERMAL SHEETS

This study was undertaken to look at the tissue distribution of certain recently described murine macrophage antigens to determine whether distinctive subsets could be found and in particular, whether these antigens would also be present on some of the dendritic cells. To do this, mouse tissue sections were stained by monoclonal antibodies to macrophage antigens (Mac-1 (M1/70), Mac-2 (M3/38), Mac-3 (M3/84) with the use of immunoperoxidase. Mac-1 was located diffusely in the cytoplasm of round cells in a high percentage of alveolar macrophages, resident peritoneal and bone marrow cells, in splenic red pulp, and in rare perivascular cells in the thymus. Mac-1 was absent in epithelial cells and Langerhans cells. Mac-2 was strongly positive in many dendritic cells in the thymic medulla, more than the cortex, in paracortex and medulla of lymph nodes, sparing the follicles, and in the marginal zone of spleen. There were a few positive cells in germinal centers. Mac-2 was located in a low percentage of bone marrow and a high percentage of resident peritoneal cells. When positive in sections, Mac-3 always showed granular cytoplasmic staining. Bone marrow showed a high percentage of cytoplasmic staining (>50%), as compared with low surface staining (<1%). It was found in hematopoietic cells, and in all endothelium, including post-capillary venules and lining of sinuses. It was probable that the resulting dendritic staining pattern for Mac-3 in paracortex of lymph nodes, white and red pulp, thymic cortex, and medulla included dendritic cells other than endothelial cells. Alveolar macrophages and Kupffer cells were positive for Mac-2 and Mac-3. Mac-3 also stained bile canaliculi. Clearly different staining patterns were found in epithelial cells for Mac-2 and Mac-3 in kidney tubules, intestinal mucosal lining, bronchi, choroid plexus, and epidermis.

Flotte, T. J., *Springer, T. A.* and Thorbecke, G. J.

American Journal of Pathology 111(1):112-124, 1983.

Other support: National Institutes of Health, National Institute of General Medical Sciences and the American Cancer Society Junior Faculty Fellowship.

From the Department of Pathology, New York University School of Medicine, New York, and the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

MICROSPECTROFLUOROMETRY BY DIGITAL IMAGE PROCESSING: MEASUREMENT OF CYTOPLASMIC pH

As the authors report here, an interface of their microspectrofluorometer with an image processing system performs microspectrofluorometric measurements in living cells by digital image processing. Fluorescence spectroscopic parameters can be measured by digital image processing directly from microscopic images of cells, and are automatically normalized for path length and accessible volume. Thus, an accurate cytoplasmic "map" of various spectroscopic parameters can be produced. The resting cytoplasmic pH of fibroblasts (3T3 cells) has been determined by measuring the ratio of fluorescein fluorescence exiting by two successive wavelengths (489 and 452 nm). Fluorescein-labeled dextran microinjected into the cells is used as a pH indicator since it is trapped in the cytoplasm but is excluded from the nucleus and other organelles. The average cytoplasmic pH is 6.83 (± 0.38). However, cytoplasmic pH exhibits a nonunimodal distribution, the lower mean pH being 6.74 (± 0.23). When 3T3 cells pinocytose medium containing fluorescein dextran, pinosomes peripheral to the nucleus exhibit a lower pH than those closer to the ruffling edge of the cell. The present image processing system is analyzed for linearity of detection, light scattering artifacts, signal to noise ratio, standard curves, and spatial resolution. The results obtained from digital image analysis are shown to be comparable to the results from standard microspectrofluorometry. The investigators also discuss several other applications of this ratio imaging technique in cell biology.

Tanasugarm, L., McNeil, P., Reynolds, G. T., and *Taylor, D. L.*

The Journal of Cell Biology 98:717-724, 1984.

Other support: National Science Foundation and the U. S. Department of Energy.

From the Center for Fluorescence Research in Biomedical Sciences, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, and Physics Department, Princeton University, Princeton, NJ.

FLUORESCENT ANALOG CYTOCHEMISTRY

Fluorescent analog cytochemistry is a new approach to elucidating the behavior, interaction and spatial organization of specific cellular components in living cells. A fluorescent analog consists of the native cellular component plus one or more covalently attached fluorescent probes, chosen for their spectral properties and/or environmental sensitivities. The technique, previously called molecular cytochemistry, takes advantage of the sensitivity of fluorescence detection methods and the specificity of fluorescently-labeled molecules. Cells containing fluorescent analogs can be analyzed by qualitative and/or quantitative fluorescence microscopy and by flow cytometry. Some of the technical and biological aspects of this method as applied to contractile proteins have been reviewed recently. In this paper, a fluorescent analog of the cytoskeletal protein actin has been characterized in detail. Advantages and disadvantages of this technique are considered here. New uses are also investigated. Perhaps the most exciting aspect of fluorescent analog cytochemistry is the combination of this method with various quantitative fluorescence techniques.

Taylor, D. L. et al.

Trends in Biochemical Sciences 9(3):88-91, 1984.

Other support: National Science

From the Center for Fluorescenc
ment of Biological Sciences, Ca

A METHOD FOR INCORPOR
ADHERENT CELLS

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McNeil, P. L., Murphy, R. F.,

The Journal of Cell Biology 98

Other support: National Institu

From the Department of Biolo
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Other support: National Science Foundation and the National Institutes of Health.

From the Center for Fluorescence Research in Biomedical Sciences, and the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh.

A METHOD FOR INCORPORATING MACROMOLECULES INTO ADHERENT CELLS

The researchers describe a simple method for loading exogenous macromolecules into the cytoplasm of mammalian cells adherent to tissue culture dishes. Culture medium was replaced by a thin layer of flourescently labeled macromolecules, the cells were harvested from the substrate by scraping with a rubber policeman, transferred immediately to ice cold media, washed, and then replated for culture. They refer to the method as "scrape-loading." Viability of cells was 50–60% immediately after scrape-loading and was 90% for those cells remaining after 24 h of culture. About 40% of adherent, well-spread fibroblasts contained fluorescent molecules 18 h after scrape-loading of labeled dextrans, ovalbumin, or immunoglobulin-G. On average, 10 dextran molecules (70,000-mol wt) were incorporated into each fibroblast by scrape-loading in 10 mg/ml dextran. The extent of loading depended on the concentration and molecular weight of the dextrans used. A fluorescent analog of actin could also be loaded into fibroblasts where it labeled stress fibers. HeLa cells, a macrophage-like cell line, J774A.1, and human neutrophils were all successfully loaded with dextran by scraping. The method of scrape-loading should be applicable to a broad range of adherent cell types, and should be useful for loading of diverse kinds of macromolecules.

McNeil, P. L., Murphy, R. F., Lanni, F., and *Taylor, D. L.*

The Journal of Cell Biology 98:1556-1564, 1984.

Other support: National Institutes of Health and the National Science Foundation.

From the Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

SELECTIVE IMMUNOCYTOCHEMICAL DETECTION OF FLUORESCENT ANALOGS WITH ANTIBODIES SPECIFIC FOR THE FLUOROPHORE

Fluorescent analogs of cellular components are finding increasing use in the field of cell biology. The power of this technique can be augmented by the use of antibodies specific for the fluorophore to visualize selectively the fluorescent analog at the electron microscope level. Rabbit antibodies specific for fluorescein were elicited and purified according to published methods (Lopatin and Voss (1971). Biochemistry 10:208). Immune sera and IgG formed precipitin lines with fluorescein-labeled proteins in Ouchterlony immunodiffusion assays and significantly quenched the fluorescence of fluorescein-labeled proteins. Immune IgG and Fab fragments decorated fluorescein-labeled actin, but not unlabeled actins, in negative-stained preparations. Anti-fluorescein IgG was used for immunofluorescent localization of fluorescein-labeled actin following microinjection of the fluorescent analog into living cells. This approach was extended to the immunoelectron microscopic localization of injected

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

analog at the subcellular level by the use of an electron-dense marker coupled to goat anti-rabbit IgG. Many other fluorescent probes also can be used as haptens for production of antibodies. Therefore, a general method for localizing fluorescently labeled molecules at the electron microscopic level is now available. Several other applications of anti-fluorescein antibody in studies involving fluorescent analogs are also suggested.

Luby-Phelps, K., Amato, P. A., and *Taylor, D. L.*

Cell Motility 4:137-149, 1984.

Other support: National Institutes of Health.

From the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh.

ANTIGEN PRESENTATION: COMMENTS ON ITS REGULATION AND MECHANISM

The essential nature of antigen presentation was not appreciated until the discovery of the restriction imposed on cellular interactions by products of the major histocompatibility gene complex (MHC). It has become apparent that an essential step in the induction of responsiveness to all protein antigens is the activation of helper T cells. There are three points about antigen presentation by murine macrophages that the authors feel should be stressed: (1) expression of Ia antigens by macrophages is not constitutive but is subject to both positive and negative regulation; (2) complex antigens must be "processed" by the macrophage to become immunogenic; and (3) antigen-presenting cells other than the classical macrophage can function in the presentation of at least some antigens. Experimental results are presented here which lead to the conclusion that the regulation of Ia expression by the macrophage may be a mechanism not only to amplify antigen presentation after the introduction of foreign antigen, but to diminish responsiveness during neonatal development and at local sites in the adult. The immune system has evolved in a manner so as to depend on the expression of Ia antigens. This allows for a sensitive control mechanism that limits antigen recognition by lymphocytes, some of which are autoreactive. The control mechanisms that are discussed here include the restriction of Ia expression to selected cells (e.g., the macrophages) and imposing on these selected cells positive and negative regulatory controls.

Unanue, E. R. et al

The Journal of Immunology 132(1):1-5, 1984.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

EVIDENCE FOR ROLE OF HYDROXYL RADICAL IN COMPLEMENT AND NEUTROPHIL-DEPENDENT TISSUE INJURY

The investigators, using their recently described model of acute lung injury in rats after systemic activation of complement by cobra venom factor (CVF), demonstrated that pretreatment of animals with human milk apolactoferrin (in its native or derivitized

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Ward, P. A. et al.

The Journal of Clinical In

Other support: National I
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From the University of M
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form), but not iron-saturated lactoferrin, provides significant protection against complement- and neutrophil-mediated lung injury. The synthetic iron chelator deferoxamine mesylate also affords protection from lung injury. The protective effects of apolactoferrin are not related to a blocking of CVF induced complement activation. They also demonstrated that infusion of ionic iron, especially Fe^{3+}, greatly potentiates lung vascular injury after systemic complement activation. Finally, protection from lung injury occurs in animals pretreated with the potent scavenger of hydroxyl radicals ($OH^{\cdot}$), dimethyl sulfoxide. Based on transmission electron microscopy, CVF-treated rats show leukoaggregates and endothelial cell destructon in interstitial pulmonary capillaries, along with intraalveolar hemorrhage and fibrin deposition. In animals protected with apolactoferrin, deferoxamine mesylate or dimethyl sulfoxide, the morphological studies reveal leukoaggregates but no endothelial cell damage, hemorrhage or fibrin deposition. These data support the concept that tissue injury that is complement and neutrophil dependent may be related to generation of $OH^{\cdot}$ derived from H_2O_2 after leukocytic activation.

Ward, P. A., et al.

The Journal of Clinical Investigation 72: 789-801, 1983.

Other support: National Institutes of Health and Veteran's Administration Merit Review.

From the University of Michigan Medical School, Ann Arbor, and the Department of Medicine, Veterans Administration Hospital, Ann Arbor.

VII. *Metabolic Studies*

THE INFRASTRUCTURE OF AORTIC ELASTIC FIBERS

In this attempt to determine whether the "amorphous" elastin component of elastic fibers possesses an internal structure that can be detected in thin sections of aldehyde-fixed tissues, the authors studied samples of rat aortas that had been fixed in glutaraldehyde-ruthenium red and osmium-ruthenium red mixtures. As they report, elastic fibers are composed of a central core of elastin that is amorphous and electron-lucent in conventional transmission electron micrographs and peripheral microfibrils. A complex infrastructure within the amorphous elastin of mature rat aorta is made visible by fixation and staining with a glutaraldehyde-ruthenium red mixture in phosphate buffer or osmium-ruthenium red in cacodylate buffer. The infrastructure is composed of at least two interlacing but distinct elastic structural components: a framework of circumferentially orientated microfibrils and a three-dimensional meshwork of filaments that permeate the fiber. The latter resembles a reticulum that has previously been observed in freeze-fractured and negatively stained elastin and attributed to the supramolecular organization of elastin. Microfibrils also extend from the core of the elastic fiber into the surrounding matrix where they appear to function as anchoring fibers. These observations indicate that the elastic properties of the arterial wall are an integrated function of both elastin and microfibrils.

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Source: https://www.industrydocuments.ucsf.edu/docs/mpxk0000

Goldfischer, S. et al.

Tissue & Cell 15(3):429-435, 1983.

Other support: National Institutes of Health.

From the Departments of Pathology and Biochemistry, Albert Einstein College of Medicine, The Bronx, NY.

HYPOPHOSPHATASIA: CLINICOPATHOLOGIC COMPARISON OF THE INFANTILE, CHILDHOOD, AND ADULT FORMS

Hypophosphatasia — a rare, heritable, metabolic bone disease — is characterized by subnormal circulating alkaline phosphatase (ALP) activity, increased blood and urine levels of phosphoethanolamine (PEA) and inorganic pyrophosphate (PPi), dental abnormalities, and defective bone mineralization that can present clinically as rickets in children or osteomalacia in adults. Depending upon the age at which skeletal disease is noted, hypophosphatasia has been classified into three forms: infantile, childhood and adult. In this study, the authors assayed ALP activity in bone tissue from patients with all forms of the hypophosphatasia (4 infants, 2 children, 17 adults). They compared the findings to the clinical and histopathologic expression of their disease in order to explore the clinical heterogeneity of this inborn error and the hypothesis that ALP is bone is important for orderly skeletal mineralization. As summarized, histochemical and direct enzyme analysis of osseous tissue from the 23 patients with hypophosphatasia revealed that all clinical forms of this inherited metabolic bone disease are characterized by deficiency of ALP activity in bone. The severe infantile form has the most profound deficiency. The findings presented here are most consistent with evidence that childhood and adult hypophosphatasia often represent clinical expression of the heterozygous state for ALP deficiency which, when homozygous, results in the clinically severe, recessive, infantile form. In general, the severity of the clinical expression of the disease reflects the magnitude of the deficiency of ALP in bone. This is the expected finding for this inborn error of metabolism, which illustrates the major role bone ALP activity has in the process of normal skeletal mineralization.

Fallon, M. D., Teitelbaum, S. L., Weinstein, R. S., *Goldfischer, S.*, Brown, D. M., and Whyte, M. P.

Medicine 63(1):12-24, 1984.

Other support: National Institutes of Health and the Shriners Hospital for Crippled Children

From the Department of Pathology and Laboratory Medicine, the Jewish Hospital of St. Louis, Washington University School of Medicine, St. Louis; Metabolic Bone Disease Laboratory, Section of Metabolic and Endocrine Disease, Department of Medicine, Medical College of Georgia, Augusta, Department of Pathology, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY; Department of Pediatric Nephrology, University of Minnesota Medical School, Minneapolis; and Division of Bone and Mineral Metabolism, Departments of Medicine and Pediatrics, Washington University School of Medicine, and Metabolic Research Unit, Shriners Hospital for Crippled Children, St. Louis

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Other support: U. S. Public H

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ASSAY OF PEPTIDASE AN

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CELL BIOLOGY OF PULMONARY ENDOTHELIUM

The complexicity and biological importance of the endothelium are discussed in this paper. As the abstract states, since the late 1960s understanding of the cell biology of endothelium has been transformed. Endothelium is not merely a metabolically inert, semipermeable barrier separating blood from parenchyma; rather, it is a layer of metabolically active cells. In lungs, even gas exchange may be assisted by reactions occurring on the endothelial surface. Endothelial cells synthesize specific proteins (some for export); these cells have receptors and enzymes capable of reacting with certain hormones and other excitatory substances as they pass in circulating blood. Endothelium is antithrombogenic unless injured; when injured, endothelium becomes thrombogenic and then thrombolytic. Endothelium may sometimes retard the development of inflammation and at other times may facilitate it. In addition to providing sites for exchange of nutrients and metabolites, endothelium interacts with prohormones and hormones to determine the composition of blood moving downstream. The latter is a key function of pulmonary endothelium: its venous effluent is systemic arterial blood. Efforts to understand how endothelium accomplishes its wide range of metabolic activities have motivated parallel efforts to define the fine structure of the endothelial cell. Thus, it has become feasible to visualize habitats of two surface enzymes, angiotensin-converting enzyme and carboxypeptidase N. Efforts to visualize surface enzymes required development of means of replicating cell surfaces, a methodology that in turn provided the first *en face* view of the glycocalyx. Given the ubiquity of vascular endothelium and its activities, it is difficult to imagine an area of medical practice that can safely ignore requirements of appropriately functioning endothelium.

Ryan, U. S. and Ryan, J. W.

Circulation 70(suppl III), III-46, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ASSAY OF PEPTIDASE AND PROTEASE ENZYMES *IN VIVO*

Means are becoming available for measuring the instantaneous activities of cell-bound peptidase and protease enzymes whose catalytic sites have access to the vascular space. For example, it is now possible to measure the effective concentration of angiotensin converting enzyme in lungs of chronically cannulated, unanesthetized experimental animals, and it is feasible to make such measurements in man. Although the text of this paper deals primarily with the assay of peptidase and protease enzyme activities, the approach and general principles described here undoubtedly can be used to measure activity of virtually any accessible hydrolase enzyme for which a suitable substrate can be found. The latter clearly includes esterase and phosphatase enzymes. Since the mid-1960s, when it became evident that the metabolic fates of certain vasoactive substances were determined within miniseconds rather than minutes, the focus of some investigators' studies has changed from hormones themselves to the enzymes that catalyze their degradation. In this in-depth consideration of an *in vivo* assay system, sections are devoted to: (1) Substrates for *in vivo* assays; (2) Methods of assay of enzymes *in vivo*; (3) Enzyme kinetics *in vivo*; (4) Mathematics of cylinders: Relevance to *in vivo* assays; (5) What is the concentration of angiotensin converting enzyme

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in a capillary? and (6) Clinical implications of *in vivo* assays. In the concluding section, the author maintains that we still know little of what kinds of information can be obtained by assaying enzyme activities *in vivo*. Yet it is difficult to avoid the conclusion that this assay technology provides a basis for understanding aspects of biochemistry in physiological terms and aspects of physiology in biochemical terms.

Ryan, J. W. (*Ryan, U.*)

Biochemical Pharmacology 32(14):2127-2137, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami, Miami, FL.

THE ENDOTHELIAL CELL SURFACE

The conclusion to this carefully considered research paper states that the endothelial surface cannot be assumed to be smooth any more than red cells can be assumed to be spheres. The surface is an ever changing one; at times it is covered with finger-like projections and is carpeted with a polymeric coat of extra-cellular materials. In addition, the endothelial surface is replete with enzymes that actively change the hormonal composition of blood, influence coagulation and fibrinolysis, and control its pH (via carbonic anhydrase). In addition to surface enzymes, endothelial receptors provide for direct cellular responses. Blood-borne signals received by surface receptors are translated into intracellular actions (including synthesis and release of active compounds, changes in cell shape, and alterations in vascular tone and permeability). During agonist-receptor binding it is likely that local differences in charge are also produced. Thus, while none of the functions of endothelium originally envisioned by His, Landis and others have been challenged by results of more recent studies, it is now clear that endothelial functions and the structural correlates of these functions are far more complex than previously recognized.

Ryan, U. S. and Ryan, J. W.

Biorrheology 21:39-56, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

VIII. *Epidemiology*

RISK FACTORS, PULMONARY FUNCTION, AND MORTALITY

In 1971, a multidisciplinary study of chronic obstructive pulmonary disease was initiated at the Johns Hopkins Medical Institutions. The study was designed with two purposes in mind: First, to assess environmental and genetic factors that contribute to

the development of airways c
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as reported here show that in th
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Menkes, H. A., *Cohen, B. H*

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York, Alan R. Liss, Inc., 198

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From The Johns Hopkins Me

TWIN ANALYSIS AS A PO
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the development of airways obstruction, and second, to develop better methods of characterizing individuals at increased risk of developing airways obstruction. Results as reported here show that in the cross sectional evaluation of 2,539 non-patient adults, 11 potential risk factors were found to be significantly associated with airways obstruction. Most important among these were age and smoking. Others included: demographic variables (gender, socioeconomic status, education); the intake of coffee and diet soda; genetic markers (protease inhibitor "PI" type, ABO type, ABH secretor status); and familial pulmonary disease. Examination of combinations of risk factors in cross sectional data indicated that some of these factors were important risk factors in cigarette smokers but less evident in never smokers. For example, ABO type, familial component, coffee drinking, and diet soda intake were related to marked differences in lung function in cigarette smokers, but had little impact in never smokers. Thus, interactions of factors must be considered when assessing risk of pulmonary dysfunction.

Menkes, H. A., *Cohen, B. H.*, Beaty, T. H., Newill, C. A., and Khoury, M. J.

In: *Genetic Epidemiology of Coronary Heart Disease: Past, Present, and Future*, New York: Alan R. Liss, Inc., 1984, pp. 501-521.

Other support: National Heart, Lung and Blood Institute.

From The Johns Hopkins Medical Institutions, Baltimore.

TWIN ANALYSIS AS A POTENTIAL TOOL FOR EXAMINING PSYCHOSOCIAL FACTORS ASSOCIATED WITH AND PRECEEDING SMOKING BEHAVIORS

Data from the young cohort of the Swedish Twin Registry are being used in an attempt to describe characteristics which distinguish among current, non- and ex-smokers prior to the development of a smoking habit versus those present after establishment of the habit (or lack of one). With twins as a sample of individuals, the psychosocial variables instability, extroversion, leisure activity, relative weight, alcohol, coffee and psychopharmaceutic drug use were examined jointly as predictors of current smoking status in multiple regression analyses. This phase was intended to replicate and expand upon earlier studies characterizing current, non- and ex-smokers. These analyses were then performed on MZ nonsmoking twin individuals who were classified on the basis of their cotwin's smoking status. Patterns of variation in the psychosocial variables across the groups of nonsmokers were similar to the pattern seen for current, ex- and nonsmokers. Characteristics in MZ nonsmokers which are predictive of their cotwins' smoking status may be interpreted as those present prior to development of a smoking habit. Selected results from these analyses are presented.

Pedersen, N. L. and *Floderus-Myrrhed, B.*

Acta Geneticae Medicae et Gemellologiae 33(3):413-424, 1984.

Department of Environmental Hygiene, Karolinska Institutet, Stockholm; and College of Human Development, Pennsylvania State University, University Park, PA.

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FINNISH TWINS REARED APART. IV. SMOKING AND DRINKING HABITS. A PRELIMINARY ANALYSIS OF THE EFFECT OF HEREDITY AND ENVIRONMENT

Data on alcohol use and smoking habits were available from the 1975 questionnaire of the entire cohort. Prior to pairwise analyses, the data of individuals were compared to that of age-sex matched groups of pairs reared together. The early separated twins had a higher alcohol consumption, while for smoking only slight differences were observed compared to twins reared together. Probandwise concordance rates were computed from smoking status (ever smoker/never smoker), alcohol use (user/nonuser) and "heavy" drinking (half-bottle of spirits on one occasion at least once a month). The following results were obtained in those pairs with the environmental dissimilarity score > 15:

Age at separation	No. of cases		Smoking		Alcohol use		"Heavy" drinking	
	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
0 - 5	18	61	0.67	0.65	0.89	0.81	0.67	0.38
0 - 10	30	95	0.67	0.68	0.80	0.81	0.67	0.33

Kaprio, J., *Koskenvuo, M.*, and Langinvainio, H.

Acta Geneticae Medicae et Gemellologiae 33(3):425-433, 1984.

Other support: Yrjö Jahnsson Foundation.

From the Department of Public Health Science, University of Helsinki, Helsinki, Finland.

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Following is a list of th
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project titles. Completed p

**PRINCIPAL INVESTIGAT
OR INSTITUTION**

LEO G. ABOOD, PH.D., *Pr
Research and Biochemist
Brain Research, Universit
Medical Center, Rochester

KENNETH B. ADLER, PH.D
fessor of Pathology, Univer
College of Medicine, Burli

JOHN J. ALBERS, PH.D., *Re
Professor of Medicine*, Uni
ington School of Medicine

HARRY N. ANTONIADES
sor of Biochemistry, Har
School of Public Health, B

THOMAS M. AUNE, PH.D.
munology, The Jewish Hosp

BERNARD M. BABIOR, M
fessor of Medicine, New E
Center Hospital, Boston.

LESLIE BAER, M.D., *Assoc
Medicine*, Columbia Univ
Physicians & Surgeons, N

SAMUEL BALK, M.D., PH
New England Deaconess H

DEAN BEFUS, PH.D., *Asso
Pathology*, McMaster Univ
Ontario, Canada.

JOEL S. BENNETT, M.D.,
sor of Medicine, Hospital
of Pennsylvania, Philadel

RICHARD J. BING, M.D.,
icine (emeritus), Univers
California School of
Angeles; *Visiting Associa
stitute of Technology; Di
mental Cardiology and Sc
ment*, Huntington Me
Institutes, Pasadena, CA.

IRA B. BLACK, M.D., *Pro
Division of Developmenta
nell University Medical C

PHYLLIS B. BLAIR, PH.
Immunology, University
Berkeley.

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Active Projects

Following is a list of the principal investigators, or institutions, of projects under way or activated in the period since the previous Report, together with the respective project titles. Completed projects are listed in a later section.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
LEO G. ABOOD, PH.D., *Professor of Brain Research and Biochemistry*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Nicotine transfer-disposition in liver cells
KENNETH B. ADLER, PH.D., *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	Airway mucin secretion: effects of products from bacteria associated with chronic bronchitis
JOHN J. ALBERS, PH.D., *Research Associate Professor of Medicine*, University of Washington School of Medicine, Seattle.	High density lipoprotein quantitation
HARRY N. ANTONIADES, PH.D., *Professor of Biochemistry*, Harvard University School of Public Health, Boston.	Biosynthesis and processing of PDGF-like polypeptides in human malignant cells in culture
THOMAS M. AUNE, PH.D., *Adjunct in Immunology*, The Jewish Hospital of St. Louis.	Interferon—activation of suppressor T cell pathways
BERNARD M. BABIOR, M.D., PH.D., *Professor of Medicine*, New England Medical Center Hospital, Boston.	Studies on the mechanism of activation of the respiratory burst in neutrophils
LESLIE BAER, M.D., *Associate Professor of Medicine*, Columbia University College of Physicians & Surgeons, New York.	Cigarette smoking in normotensive and hypertensive subjects: blood pressure, renin, aldosterone and catecholamine responses
SAMUEL BALK, M.D., PH.D., *Pathologist*, New England Deaconess Hospital, Boston.	Serum mitogens, hormones, ions, viral transforming genes and tumor reversal in appropriate and autonomous initiation of cell replication
DEAN BEFUS, PH.D., *Associate Professor of Pathology*, McMaster University, Hamilton, Ontario, Canada.	The mast cell in interstitial pulmonary fibrosis
JOEL S. BENNETT, M.D., *Associate Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia.	Characterization of the platelet fibrinogen receptor
RICHARD J. BING, M.D., *Professor of Medicine (emeritus)*, University of Southern California School of Medicine, Los Angeles; *Visiting Associate*, California Institute of Technology; *Director of Experimental Cardiology and Scientific Development*, Huntington Medical Research Institutes, Pasadena, CA.	Lipoproteins and the arterial wall
IRA B. BLACK, M.D., *Professor and Chief, Division of Developmental Neurology*, Cornell University Medical College, New York.	Nicotine and neuronal development
PHYLLIS B. BLAIR, PH.D., *Professor of Immunology*, University of California, Berkeley.	Regulation of natural killer cell activity

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
THOMAS R. BROKER, PH.D., *Associate Professor of Biochemistry*, University of Rochester School of Medicine, Rochester, NY.	Cellular transformation by papilloma virus recombinants
VINCENZO BUONASSISI, M.D., *Senior Scientist and Deputy Director*, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY.	Heparan sulfate proteoglycans and blood homeostatic mechanisms
	Proteoglycans and nonthrombogenic properties of endothelial cells
DAVID L. BUSBEE, PH.D., *Professor of Toxicology*, Texas A & M University College of Veterinary Medicine, College Station.	Polynuclear aromatic hydrocarbon transport by serum lipoproteins
EDWARD J. CAMPBELL, M.D., *Assistant Professor of Medicine*, Washington University School of Medicine, St. Louis.	Modulators of inflammatory cell proteolytic activity
FRANCIS C. CHAO, M.D., PH.D., *Senior Investigator*, Center for Blood Research, Boston.	Platelet activation and blood hypercoagulability
LAN BO CHEN, PH.D., *Associate Professor of Pathology*, Sidney Farber Cancer Institute, Boston.	Studies on human oat cell carcinomas
YUAN-TSONG CHEN, M.D., PH.D., *Assistant Professor of Pediatrics*, Duke University Medical Center, Durham, NC.	Recombinant DNA approaches to assess risk for lung cancer
DOUGLAS BROCK CINES, M.D., *Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia.	Immune injury of human endothelial cells
JAN F. CHLEBOWSKI, PH.D., *Assistant Professor of Biochemistry*, Medical College of Virginia, Richmond.	Calorimetric investigation of proteinase-α_2 macroglobulin interaction
CURTI. CIVIN, M.D., *Assistant Professor of Oncology & Pediatrics*, The Johns Hopkins Oncology Center, Baltimore.	Biochemistry and function of human granulopoietic antigens
BRIAN L. CLEVINGER, PH.D., *Assistant Professor of Biomedical Science*, Washington University School of Dental Medicine, St. Louis.	Role of J segment in V segment expression
CHARLES G. COCHRANE, M.D., *Member, Department of Immunopathology*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mediation systems in inflammatory lung disease
BERNICE H. COHEN, PH.D., *Professor and Director, Human Genetics/Genetic Epidemiology Program*, The Johns Hopkins University School of Hygiene and Public Health, Baltimore.	Genetic-epidemiologic characteristics of smokers and nonsmokers
ROBERT W. COLMAN, M.D., *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Initiation of plasma coagulation and kinin forming systems in man

PRINCIPAL INVESTIGATOR OR INSTITUTION
EVA BROWN CRAMER, PH.D., *Professor of Anatomy and Cell* Downstate Medical Center, Broo
CARL E. CRUETZ, PH.D., *Assista sor of Pharmacology*, Universit ginia School of Medicine, Charl
GIDON CZAPSKI, M.Sc., PH.D., *of Physical Chemistry*, The Hebre sity, Jerusalem, Israel.
IVAN DAMJANOV, M.D., PH.D *sor of Pathology*, Hahnemann School of Medicine, Philadelphi
ALBERT B. DEISSEROTH, M.D *Professor of Medicine*, Veterans tration Medical Center, San Fra
PETER H. DUESBERG, PH.D., *P Molecular Biology*, University nia, Berkeley.
HAROLD F. DVORAK, M.D., *partment of Pathology*, Beth Isr tal, Boston.
V. GENE ERWIN, PH.D., *Profess macology; Dean*, University o School of Pharmacy, Boulder.
ALVAN R. FEINSTEIN, M.D., *P Medicine & Epidemiology*, Yale School of Medicine, New Have
RICHARD FENTON, PH.D., *In Physiology*, University of Ma School of Medicine, Worcester
THOMAS H. FINLAY, PH.D., *Professor of Obstetrics and C* New York University Medical C York.
PAUL B. FISHER, PH.D., *Senio Associate, Department of Mi* Columbia University College of & Surgeons, New York.
JOSEPH A. FONTANA, M.D., *sistant Professor of Medicine a istry*, West Virginia Universi Center, Morgantown.
JUDITH ANN FOSTER, PH.D. *and Chairperson, Department* Syracuse University, Syracuse
RICHARD B. FOX, M.D., *Assis sor of Pediatrics*, Children's H poration, Boston.
AARON E. FREEMAN, PH.D., *tist*, California Biomedical Res dation, La Jolla, CA.

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
EVA BROWN CRAMER, PH.D., *Associate Professor of Anatomy and Cell Biology*, Downstate Medical Center, Brooklyn, NY.	Studies of inflammation using an *in vitro* model
CARL E. CRUETZ, PH.D., *Assistant Professor of Pharmacology*, University of Virginia School of Medicine, Charlottesville.	Role of protein phosphorylation in nicotine-induced catecholamine release
GIDON CZAPSKI, M.Sc., PH.D., *Professor of Physical Chemistry*, The Hebrew University, Jerusalem, Israel.	On the toxicity of oxygen and superoxide ion: is superoxide toxic?
IVAN DAMJANOV, M.D., PH.D., *Professor of Pathology*, Hahnemann University School of Medicine, Philadelphia.	Developmentally pluripotent human lung cancer stem cells
ALBERT B. DEISSEROTH, M.D., PH.D., *Professor of Medicine*, Veterans Administration Medical Center, San Francisco.	Study of altered alpha globin genes in leukemia and solid tumors.
PETER H. DUESBERG, PH.D., *Professor of Molecular Biology*, University of California, Berkeley.	Transforming genes of two acute leukemia viruses.
HAROLD F. DVORAK, M.D., *Chief, Department of Pathology*, Beth Israel Hospital, Boston.	Pathogenesis of tumor desmoplasia
V. GENE ERWIN, PH.D., *Professor of Pharmacology; Dean*, University of Colorado School of Pharmacy, Boulder.	Effects of nicotine on neuropeptide secretion by intact mouse brain, a pharmacogenetic study
ALVAN R. FEINSTEIN, M.D., *Professor of Medicine & Epidemiology*, Yale University School of Medicine, New Haven, CT.	Smoking, detection bias and primary lung cancer
RICHARD FENTON, PH.D., *Instructor in Physiology*, University of Massachusetts School of Medicine, Worcester.	Physiological effects of nicotine on calcium and adenosine metabolism by the heart
THOMAS H. FINLAY, PH.D., *Associate Professor of Obstetrics and Gynecology*, New York University Medical Center, New York.	Structure, properties and regulation of mouse plasma protease inhibitors
PAUL B. FISHER, PH.D., *Senior Research Associate, Department of Microbiology*, Columbia University College of Physicians & Surgeons, New York.	Chemical-viral interactions in cell transformation
JOSEPH A. FONTANA, M.D., PH.D., *Assistant Professor of Medicine and Biochemistry*, West Virginia University Medical Center, Morgantown.	Glycosyltransferases and glycoprotein synthesis in differentiation induced phenotypic reversal of malignancy by retinoic acid cyclic nucleotides and other agents
JUDITH ANN FOSTER, PH.D., *Professor and Chairperson, Department of Biology*, Syracuse University, Syracuse, NY.	Involvement of elastin in lung disease
RICHARD B. FOX, M.D., *Assistant Professor of Pediatrics*, Children's Hospital Corporation, Boston.	Role of glycosaminoglycans in lung edema
AARON E. FREEMAN, PH.D., *Staff Scientist*, California Biomedical Research Foundation, La Jolla, CA.	Rodent and human lung epithelial cell culture as a tool for carcinogenesis research *in vitro*

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ERROL C. FRIEDBERG, M.D., *Associate Professor of Pathology*, Stanford University, Stanford, CA.	Complementing human cells with cloned yeast DNA repair genes
KJELL FUXE, M.D., *Professor of Histology*, The Karolinska Institute, Stockholm.	Nicotine, catecholamines and neuroendocrine functions
JAMES W. GAUBATZ, PH.D., *Assistant Professor of Biochemistry*, University of South Alabama, Mobile.	Direct demonstration of high affinity drug-DNA interactions by restriction enzyme mapping
JI BERNARD L. GEE, M.D., *Professor of Medicine*, Yale University School of Medicine, New Haven, CT.	Tissue matrix and phagocyte injury: relative contributions of proteases and oxidants
JACQUES E. GIELEN, PH.D., *Associate Professor, Laboratory of Medical Chemistry, Toxicology and Hygiene*, Institute of Pathology, University of Liège, Liège, Belgium.	Towards a molecular understanding of mono-oxygenase regulatory mechanisms in animals and man
GORDON NELSON GILL, M.D., *Professor of Medicine*, University of California, San Diego, La Jolla.	Epidermal growth factor receptor gene in epidermoid carcinoma
GABRIEL C. GODMAN, M.D., *Professor of Pathology*, Columbia University College of Physicians & Surgeons, New York.	Cytoskeletal organization of the endothelial cell:in regulation of shape contractility and surface movement
WARREN M. GOLD, M.D., *Professor of Medicine*, Cardiovascular Research Institute, University of California, San Francisco.	Effect of ozone on airway mast cells.
SIDNEY GOLDFISCHER, M.D., *Professor of Pathology*, Albert Einstein College of Medicine, The Bronx, NY.	Extracellular matrix-cytochemistry and ultrastructure
MAURICE GREEN, M.D., *Director, Institute for Molecular Virology*, St. Louis University Medical Center, St. Louis.	Amplification of human adenovirus transformation proteins in prokaryotic and eukaryotic cells
CHARLES S. GREENBERG, M.D., *Assistant Professor of Medicine*, Duke University Medical Center, Durham, NC.	Transglutaminases and atherosclerosis
MARK I. GREENE, PH.D., *Associate Professor of Pathology*, Harvard Medical School, Boston.	Suppressor cells in syngeneic tumor immunity
NOBUYOSHI HAGINO, M.D., PH.D., *Professor of Anatomy*, University of Texas Health Science Center, San Antonio.	Nicotinic receptors of LHRH axon terminals in the median eminence
CAROLINE B. HALL, M.D., *Associate Professor of Pediatrics and Medicine*, University of Rochester School of Medicine, Rochester, NY.	Interrelationship of infectious lower respiratory tract disease in infancy, and host and environmental factors to later development of chronic lung disease
LINDA M. HALL, PH.D., *Associate Professor of Genetics and Neuroscience*, Albert Einstein College of Medicine of Yeshiva University, The Bronx, NY.	Genetic differences in nicotine sensitivity in *Drosophila melanogaster* strains.

PRINCIPAL INVESTIGATOR OR INSTITUTION
PAUL HAMOSH, M.D., *Associ of Physiology and Biophysic cine*, Georgetown University Medicine and Dentistry, Wash
RONALD G. HARVEY, PH.D. *Organic Chemistry*, The U Chicago.
ROBERT M. HOFFMAN, PH *Professor of Pediatrics in Re* versity of California School La Jolla.
WAYNE HOSS, PH.D., *Associ* Center for Brain Research, Rochester Medical Center, R
HAROLD P. JONES, PH.D., *fessor of Biochemistry*, Univ Alabama, Mobile.
MORRIS J. KARNOVSKY, M *Shattuck Professor of Path omy*, Harvard Medical Scho
SIMON KARPATKIN, M.D. *Medicine*, New York Univ Center, New York.
SHIRLEY L. KAUFFMAN, M *of Pathology*, State Universit Downstate Medical Center,
INGEGERD M. KEITH, PH *Professor of Anatomy*, Uni consin School of Veterin Madison.
HEINZ KOHLER, M.D., PH *Department of Molecular* Roswell Park Memorial Ins NY.
MARKKU KOSKENVUO, M *and Chairman, Departm Health Science*, Universit Helsinki, Finland.
ROBERT W. KREILICK, PH *of Chemistry*, University Rochester, NY.
KLAUS E. KUETTNER, PH *and Chairman, Departmer try*, Rush College of Heal Rush Medical College, Ru St. Luke's Medical Center
ABEL LAJTHA, PH.D., *Dire* State Research Institute for and Drug Addiction, New
DON LAPENAS, M.D., *As of Pathology*, University lege of Medicine, Burlingt
E. CLINTON LAWRENCE, *Professor of Medicine*, B Medicine, Houston.

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
PAUL HAMOSH, M.D., *Associate Professor of Physiology and Biophysics, and Medicine*, Georgetown University Schools of Medicine and Dentistry, Washington, D.C.	Cigarette smoke and lipoprotein remodeling by the lung
RONALD G. HARVEY, PH.D., *Professor of Organic Chemistry*, The University of Chicago.	Novel anticarcinogenic coumarins and flavones
ROBERT M. HOFFMAN, PH.D., *Assistant Professor of Pediatrics in Residence*, University of California School of Medicine, La Jolla.	Methionine dependence, methylation and organic transformation
	Regulation of cellular oncogenes
WAYNE HOSS, PH.D., *Associate Professor*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Studies of nicotine interaction with blood cells
HAROLD P. JONES, PH.D., *Assistant Professor of Biochemistry*, University of South Alabama, Mobile.	Calcium-dependent regulatory proteins and neutrophil activation
MORRIS J. KARNOVSKY, M.B., B. CH., *Shattuck Professor of Pathological Anatomy*, Harvard Medical School, Boston.	The molecular basis of pulmonary surfactant secretion by type II pneumocytes: studies in intact cells and a cell-free system
SIMON KARPATKIN, M.D., *Professor of Medicine*, New York University Medical Center, New York.	The role of platelets in tumor cell metastases
SHIRLEY L. KAUFFMAN, M.D., *Professor of Pathology*, State University of New York Downstate Medical Center, Brooklyn.	Oncogenes in chemical carcinogenesis
INGEGERD M. KEITH, PH.D., *Assistant Professor of Anatomy*, University of Wisconsin School of Veterinary Medicine, Madison.	Part I: Lung neuroendocrine cell innervation
	Part II: Transplacental effect of smoking on lung neuroendocrine cells in the neonate
HEINZ KOHLER, M.D., PH.D., *Director, Department of Molecular Immunology*, Roswell Park Memorial Institute, Buffalo, NY.	Multi-targeting with hybridomas on tumor cells
MARKKU KOSKENVUO, M.D., *Professor and Chairman, Department of Public Health Science*, University of Helsinki, Helsinki, Finland.	The Finnish Twin Cohort Follow-up Study
ROBERT W. KREILICK, PH.D., *Professor of Chemistry*, University of Rochester, Rochester, NY.	Investigations of the interaction of nicotine with membranes
KLAUS E. KUETTNER, PH.D., *Professor and Chairman; Department of Biochemistry*, Rush College of Health Sciences and Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago.	Regulation of proliferation of invasive cells
ABEL LAJTHA, PH.D., *Director*, New York State Research Institute for Neurochemistry and Drug Addiction, New York.	Genetic basis for nicotine response
DON LAPENAS, M.D., *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	The association of inorganic dust deposition with pulmonary neoplasia in tobacco users
E. CLINTON LAWRENCE, M.D., *Assistant Professor of Medicine*, Baylor College of Medicine, Houston.	Effects of cigarette smoking on immunoglobulin production by human bronchial lymphocytes

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
JOSEPH D. LOCKER, M.D., PH.D., *Assistant Professor of Pathology and Biochemistry*, University of Pittsburgh School of Medicine.	DNA methylation in neoplasia
GESINA L. LONGNECKER, PH.D., *Associate Professor of Pharmacology*, University of South Alabama College of Medicine, Mobile.	Studies of platelet and endothelial prostanoid production as possible cardiovascular risk indicators in smokers
RONALD B. LUFTIG, PH.D., *Professor and Head, Department of Microbiology and Immunology*, LSU Medical Center, New Orleans.	Interactions between RNA viruses and chemical carcinogens
JAN M. LUNDBERG, M.D., *Assistant Professor of Pharmacology*, Karolinska Institutet, Stockholm, Sweden.	Sensory neuropeptides and smoke-induced irritation in the respiratory tract
HENRY T. LYNCH, M.D., *Professor and Chairman, Department of Preventive Medicine and Public Health*, Creighton University School of Medicine, Omaha.	Genetic and biomarker studies of cancers of the respiratory tract, pancreas and urinary bladder
BRUCE A. MACHER, PH.D., *Assistant Professor of Pharmaceutical Chemistry*, University of California, San Francisco.	Chemistry and biology of complex carbohydrates
HOWARD S. MAKER, M.D., *Associate Professor of Neurology*, Mount Sinai School of Medicine, New York.	Nicotine action on brain neurotransmitters and in an animal model of Parkinson's disease
RICHARD A. MARKHAM, M.D., *Assistant Professor of Medicine and of Microbiology and Immunology*, The Jewish Hospital of St. Louis.	T cell-mediated immunity to *Pseudomonas aeruginosa*
ALAN C. McLAUGHLIN, PH.D., *Lecturer in Biochemistry Biophysics*, University of Pennsylvania School of Medicine, Philadelphia.	Interaction of divalent cations with model and biological membranes
STELLA MITRANI-ROSENBAUM, PH.D., *Professor of Virology*, Hebrew University-Hadassah Medical School, Jerusalem, Israel.	Molecular analysis of human genital papilloma virus
FERID MURAD, M.D., PH.D., *Professor of Medicine and Pharmacology*, Stanford University, and *Chief of Medicine*, Palo Alto V.A. Hospital, Stanford, CA.	Mechanism of nitric oxide activation of guanylate cyclase Role of cyclic GMP in smooth muscle relaxation
CHRISTOPHER MURLAS, M.D., *Assistant Professor of Medicine*, University of Cincinnati.	Electromechanical properties of airway muscle
JAY A. NADEL, M.D., *Professor of Medicine, Physiology and Radiology*, Cardiovascular Research Institute, University of California, San Francisco.	Mechanisms of airway hyperreactivity
SUSAN L. NAYLOR, PH.D., *Associate Professor of Human Genetics*, The University of Texas Health Science Center, San Antonio.	Molecular and genetic analysis of small cell lung cancer
DONALD J. NELSON, PH.D., *Associate Professor of Chemistry*, Clark University, Worcester, MA.	Calmodulin interactions with target proteins and synaptic vesicles

PRINCIPAL INVESTIG/ OR INSTITUTION
STEFAN NIEWIAROWSI *Professor of Physiology* search Center, Temple U Medicine, Philadelphia.
JANET M. OLIVER, PH *Pathology*, University School of Medicine, Alb
F. WILLIAM ORR, M.D., *sor of Pathology*, Unive Winnipeg, Manitoba, Ca
YOSHIO OSAWA, PH.D. *Biochemistry Departme* dation of Buffalo, Buffa
BENEDICHT U. PAULI, D *Professor of Pathology*, I St. Lukes Medical Cente
BORIS M. PETERLIN, M *fessor of Medicine*, Se tology-Clinical Immunol California School of Me cisco.
DENNIS R. PETERSEN, *Professor of Pharmacol* Colorado School of Phar
JULIA M. POLAK, D.Sc *Lecturer in Histopathol* graduate Medical Scho Hospital, London.
WILLIAM A. PRYOR, PH *sor of Chemistry*, Louisi sity, Baton Rouge.
MICHAEL S. RABSON, *Scientist, Laboratory o* tional Cancer Institute, E
TIMOTHY J. REGAN, M *Medicine; Director, Divi cular Diseases*, College Dentistry of New Jersey, cal School, Newark.
JOHN E. REPINE, M.D., *Webb-Waring Lung In Professor of Medicine*; U rado Health Sciences Ce
ROBERT RESNICK, M.D *fessor of Reproductive* sity of California Med Diego.

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
STEFAN NIEWIAROWSKI, M.D., PH.D., *Professor of Physiology*; Thrombosis Research Center, Temple University School of Medicine, Philadelphia.	Platelet interaction with fibrinogen and its significance in hemostasis
JANET M. OLIVER, PH.D., *Professor of Pathology*, University of New Mexico School of Medicine, Albuquerque.	Regulation of the membrane oxidase of human polymorphonuclear leukocytes
F. WILLIAM ORR, M.D., *Associate Professor of Pathology*, University of Manitoba, Winnipeg, Manitoba, Canada.	Role of local factors in pulmonary metastasis
YOSHIO OSAWA, PH.D., *Head, Endocrine Biochemistry Department*; Medical Foundation of Buffalo, Buffalo, NY.	Aromatase inhibitors in cigarette smoke and tobacco
BENDICHT U. PAULI, D.V.M., *Associate Professor of Pathology*, Rush Presbyterian-St. Lukes Medical Center, Chicago.	Local regulation of tumor invasion by host-derived proteinase inhibitors
BORIS M. PETERLIN, M.D., *Assistant Professor of Medicine*; Section of Rheumatology-Clinical Immunology, University of California School of Medicine, San Francisco.	Biology and molecular biology of the differentiation of a human monocytoid cell line
DENNIS R. PETERSEN, PH.D., *Associate Professor of Pharmacology*, University of Colorado School of Pharmacy, Boulder.	Implementation of the isolated perfused liver to study nicotine metabolism and metabolic interactions
JULIA M. POLAK, D.SC., M.D., *Senior Lecturer in Histopathology*, Royal Postgraduate Medical School, Hammersmith Hospital, London.	Investigation of the role of regulatory peptides in human lung disease
WILLIAM A. PRYOR, PH.D., *Boyd Professor of Chemistry*; Louisiana State University, Baton Rouge.	Free radical chemistry of cigarette smoke
MICHAEL S. RABSON, PH.D., *Research Scientist, Laboratory of Pathology*; National Cancer Institute, Bethesda, MD.	Analysis of sequences required for bovine papilloma virus transformation and autonomous plasmid replication
TIMOTHY J. REGAN, M.D., *Professor of Medicine; Director, Division of Cardiovascular Diseases*, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark.	Susceptibility of arrhythmias and catecholamine metabolism in chronic smoking animals
JOHN E. REPINE, M.D., *Assistant Director, Webb-Waring Lung Institute*; *Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	Basic mechanisms of lung injury from inhaled oxidants
ROBERT RESNICK, M.D., *Associate Professor of Reproductive Medicine*, University of California Medical Center, San Diego.	The effect of nicotine on uterine and fetal cardiovascular hemodynamics

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
VIRGINIA L. RICHMOND, PH.D., *Research Associate*, Pacific Northwest Research Foundation, Seattle.	Elastic fiber microfibrillar protein structure
EUGENE ROBERTS, PH.D., *Research Scientist*, City of Hope Research Institute, Duarte, CA.	Effects of nicotine on the cerebrovasculature *in vitro*
PETER M. ROSS, PH.D., *Research Associate*, The Rockefeller University, New York.	DNA damage and selective maintenance of animal genes
UNA S. RYAN, PH.D., *Research Professor of Medicine*, University of Miami School of Medicine, Miami, FL.	Interactions of hormones with cells of the pulmonary vascular wall
BRAHMA P. SANI, PH.D., *Head, Protein Biochemistry*, Southern Research Institute, Birmingham, AL.	Selenium-binding proteins
REGINA M. SANTELLA, PH.D., *Associate Professor of Medicine and Environmental Sciences*, Columbia University, New York.	Development of monoclonal antibodies to carcinogen-DNA adducts
B. V. RAMA SASTRY, D.SC., PH.D., *Professor of Pharmacology*, Vanderbilt University School of Medicine, Nashville, TN.	Maternal smoking and blood concentrations of amino acids in umbilical arteries and veins Influence of nicotine on the release of acetylcholine in the human placenta and its implications on the fetal growth
ISHAIAHU SCHECHTER, PH.D., *Senior Lecturer in Biochemistry*, The George S. Wise Faculty for Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Effect of thiols and disulfides on cholesterol metabolism
CHARLES H. SCOGGIN, M.D., *Head, Division of Clinical Applications; Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	The somatic cell genetics of lung cancer
HENRY SERSHEN, PH.D., *Research Scientist IV, Neurochemistry Division*, Nathan S. Kline Institute, Ward's Island, New York.	Development of an animal model of Parkinson's disease
ROBERT J. SKLAREW, PH.D., *Research Associate Professor of Pathology*, New York University Research Service, Goldwater Memorial Hospital, Roosevelt Island, New York	Cytokinetics of heteroploid subpopulations by imaging

PRINCIPAL INVESTI OR INSTITUTION
STEVEN S. SMITH, F *search Scientist*, City stitute, Duarte, CA.
TIMOTHY A. SPRINGI *Professor of Patholog of Membrane Immu* Farber Cancer Institut
ERIC J. STANBRIDGI *Professor of Microbi* California, Irvine.
NORMAN C. STAUB, *Physiology*, Cardiova tute, University of C cisco.
DANIEL STEINBERG, *fessor of Medicine; He bolic Disease*, The Ur at San Diego, La Jolla
THOMAS P. STOSSEL *cal Oncology Unit*, M Hospital, Boston.
FLEUR L. STRAND, Pl *ology*, New York Uni
D. LANSING TAYLOR *Biology*, Carnegie- Pittsburgh.
JOSEPH CHARLES TA *ciate Research Scient* search Institute, Duar
JOHN A. THOMPSO *Professor of Pharm* University of Color macy, Boulder.
JAMES TRAVIS, PH.I *chemistry*, The Un Athens.
EMIL R. UNANUE, *Professor of Immun* Medical School, Bos
HAROLD E. VARMU *Microbiology and Im* of California, San Fr

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etics of lung cancer

animal model of Parkin-

oploid subpopulations by

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
STEVEN S. SMITH, PH.D., *Assistant Research Scientist*, City of Hope Research Institute, Duarte, CA.	Selectivity of DNA methylation in normal and oncogenically transformed cells
TIMOTHY A. SPRINGER, PH.D., *Assistant Professor of Pathology; Chief, Laboratory of Membrane Immunochemistry*, Sidney Farber Cancer Institute, Boston.	Studies of macrophage subpopulations and differentiation using monoclonal antibodies
ERIC J. STANBRIDGE, PH.D., *Associate Professor of Microbiology*, University of California, Irvine.	Transfer of specific individual human chromosomes to recipient cells
NORMAN C. STAUB, M.D., *Professor of Physiology*, Cardiovascular Research Institute, University of California, San Francisco.	Alveolar-airway barrier permeability to liquid and macromolecules in dog and sheep lung
DANIEL STEINBERG, M.D., PH.D., *Professor of Medicine; Head, Division of Metabolic Disease*, The University of California at San Diego, La Jolla.	Arterial degradation of low density lipoproteins *in vivo*
THOMAS P. STOSSEL, M.D., *Chief, Medical Oncology Unit*, Massachusetts General Hospital, Boston.	Functional anatomy of the lung macrophage
FLEUR L. STRAND, PH.D., *Professor of Biology*, New York University, New York.	Prenatal and postnatal effects of nicotine and ACTH peptides on neuromuscular development and motor behavior in rats
D. LANSING TAYLOR, PH.D., *Professor of Biology*, Carnegie-Mellon University, Pittsburgh.	Chemotaxis of macrophages
JOSEPH CHARLES TAYLOR, PH.D., *Associate Research Scientist*, City of Hope Research Institute, Duarte, CA.	Ceruloplasmin abnormality in chronic obstructive pulmonary disease
JOHN A. THOMPSON, PH.D., *Associate Professor of Pharmaceutical Chemistry*, University of Colorado School of Pharmacy, Boulder.	Chromatographic separation and comparative metabolism of d- and l-nicotine
JAMES TRAVIS, PH.D., *Professor of Biochemistry*, The University of Georgia, Athens.	Proteolytic enzymes and inhibitors in emphysema
EMIL R. UNANUE, M.D., *Mallinckrodt Professor of Immunopathology*, Harvard Medical School, Boston.	Physiopathology of normal and activated macrophages
HAROLD E. VARMUS, M.D., *Professor of Microbiology and Immunology*, University of California, San Francisco.	Functional analysis of cellular oncogenes activated during tumorigenesis

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
STEPHEN F. VATNER, M.D., *Associate Professor of Medicine*, Harvard Medical School, New England Regional primate Research Center, Southboro, MA.; *Associate in Medicine*, Peter Bent Brigham Hospital, Boston.	Direct effects of nicotine on brain circulation.
HELEN VAN VUNAKIS, PH.D., *Professor of Biochemistry*, Brandeis University, Waltham, MA.	Purification and properties of a soluble NAD(P) glycohydrolase isolated from the sponge, *M. prolifera*
EVELYN WALDSTEIN, PH.D., *Senior Lecturer*, Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel.	Repair activities for O^6-methylguanine DNA adducts in human lymphocytes of smokers *vs.* nonsmokers
PETER N. WALSH, PH.D., *Professor of Medicine*, Temple University School of Medicine, Philadephia.	Interaction of platelets with coagulation factors IX and X
PETER A. WARD, M.D., *Professor and Chairman, Department of Pathology*, The University of Michigan, Ann Arbor.	Oxygen-derived free radicals, immune complexes and tissue injury
THOMAS E. WEBB, PH.D., *Professor of Physiological Chemistry*, Ohio State University College of Medicine, Columbus.	Di-O-acetyl-D-glucarodilactone inhibition of chemical carcinogenesis
GEORGE WEINBAUM, PH.D., *Assistant Chairman for Research, Department of Medicine*, The Graduate Hospital, Philadelphia.	The role of peptide methionine sulfoxide reductase in human lungs: a possible defense against protein oxidation and elastin degradation in smokers
SAMUEL B. WEISS, PH.D., *Professor of Biochemistry and Microbiology*, The University of Chicago, Chicago.	Sequence modifications in viral DNA by benzo(α)pyrene metabolites
MICHAEL J. WELSH, M.D., *Assistant Professor of Medicine*, University of Iowa College of Medicine, Iowa City.	Mechanisms controling ion transport in airway epithelia
ÅKE WENNMALM, M.D., *Associate Professor of Clinical Physiology at Karolinska Institute*, Huddinge Hospital, Huddinge, Sweden.	Nicotine as inhibitor of prostaglandin formation; localization of the inhibitory step and characterization of the cardiovascular implications
PAUL V. WOOLEY, III, M.D., *Professor of Medicine and Pharmacology*, Georgetown University Medical Center, Washington, DC.	Effects of chemical carcinogens upon gene loci in the pancreas
STANLEY YACHNIN, M.D., *Professor of Medicine and Chief, Section of Hematology/Oncology*, The University of Chicago Medical Center, Chicago.	Models for the pathogenesis of atherosclerosis: A) biological effects of oxygenated sterol compounds; B) mevalonic acid and cholesterol biosynthesis and the biosynthesis and regulation of cell growth.

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Following is a list of the principal investigators, or institutions, of projects that have been completed prior to the period covered in this Report. Several of the individuals named are deceased. The titles and affiliations listed were those in effect at the time the work was completed.

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